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A MEMORIAL TO MILTON SILVERMAN
in Recognition of his Contributions to Biochemistry and Microbiology

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TRANSMISSION OF DEMODEX CANIS LEYDIG TO PUPS[1]

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ABSTRACT. Demodex canis was successfully transmitted in skin scraping suspensions applied to the foreheads of seven pups (5 hours to 4 weeks old) and by confining three pups (10-11 weeks old) with pups exhibiting severe demodectic mange. Nine pups from the same four litters were also reared by hand, not exposed to D. canis, and were free of mites. Eight pups, reared by dams with demodectic mange, became infested with D. canis.

Prenatal transmission of D. canis did not occur in two litters delivered from dams with demodectic mange. Five pups removed from fetal membranes and reared in isolation had no mites in their skin at necropsy while three siblings reared by their dams were infested with D. canis.

Peroral transmission was not successful when mites were fed to three pups 3 weeks old.

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Several theories have been proposed concerning the transmission of D. canis from one dog to another. Cánepa and da Graña (1945) were the leading proponents of peroral transmission. Prenatal transmission (in utero) was suggested by Kirk (1950) and Rubin (1957). Unsworth (1946) and Enigk (1949) presented data from experiments designed to demonstrate transmission of D. canis by direct application of mites to canine skin.

The difficulty of evaluating transmission attempts has plagued all researchers on this problem. Successful transmission rarely produces visible skin lesions. Often careful examination of large areas of skin is necessary to detect small populations of D. canis.

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METHODS AND MATERIALS

Puppies, to be reared by hand, were placed individually in compartmented incubators. Each compartment was 1 foot square in area. Heat was provided by thermostatically regulated heat lamps. The pups were fed a bitch milk replacement, Esbilac.[5] Each pup had its own bottle and care was taken to prevent exposing the pups to D. canis from the handler's clothing or skin.

Preliminary examination for mites was made by skin scraping, as outlined in detail by Benbrook and Sloss (1961). If no mites were found by the skin scraping technique, skin samples were taken by biopsy or at necropsy and examined by the following KOH method adapted from French (1962):

1. Tissue samples and 10 ml of 6% potassium hydroxide were placed in 12 ml conical centrifuge tubes.
2. These were incubated at 75°C until macerated, but no longer then 24 hours.
3. The tissue suspensions were centrifuged (500X gravity) for at least 3 minutes.
4. The supernatant was decanted to 0.2 ml with curved, suction pipette as illustrated in Figure 1.
5. 8 ml of "acid chloral hydrate" (40 g chloral hydrate, 25 ml distilled water, and 2.5 ml hydrochloric acid) were added.
6. The tubes were again incubated at 75°C for 4-12 hours and then centrifuged as in step 3.
7. The sediment was pipetted and placed on a glass slide, covered with 22 x 40 mm cover glass and examined with phase-contrast microscope at 100X.

INVESTIGATIONS

Dermal transmission

Several authors (Wernicke and Stolte, 1923; Nörr, 1934; Rubin, 1957; Koutz, 1957) have reported no establishment of D. canis when mites were placed on the skin of dogs. Unsworth (1946) and Enigk (1949) reported successful transmission to pups less than three months old, either by placing mites directly on the skin or when pups lived in close association with dogs having demodectic mange.

We attempted dermal transmission of D. canis to pups by direct application of mites in skin scraping suspensions or by rearing the pups with dams having demodectic mange. Skin scrapings from dogs heavily infested with D. canis were suspended in saline or tap water. A suspension containing D. canis was placed directly on a pup's skin and covered with gauze affixed with adhesive tape. If the pup was more than 1 week old, the hair was clipped before application of the mites.

[5] Trade name, The Borden Company, 350 Madison Avenue, New York 17, New York.

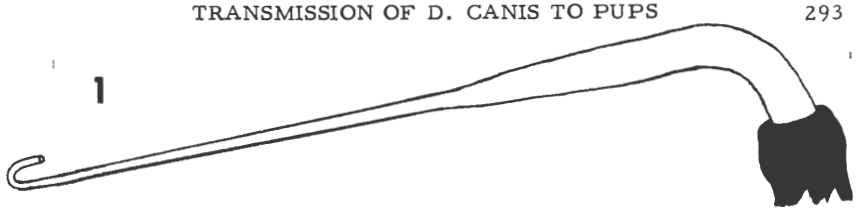

Figure 1. Curved pipette used for decanting without disturbing sediment.

Litter I

A pregnant beagle was negative for D. canis when repeatedly examined by skin scraping. Six pups were taken from her by Cesarean section. One pup was born dead and no mites were found in its skin samples when examined by the KOH method. The remaining five pups were reared in an incubator. Suspensions containing D. canis were applied to the foreheads of two pups, 24 hours old. D. canis were found easily by scraping the forehead skin of these two pups 9 weeks after application of the mites. No skin lesions were visible on the two pups at 3 months of age. However, the mite populations had established and, at necropsy, mites were found in skin samples from the eyelid, forehead, cheek, shoulder and hip of each pup.

The remaining 3 pups of Litter I were reared in isolation. No mites were found in forehead skin samples (1-1.5 square cm in area) taken by biopsy from each at 9 weeks of age. At 3 months, no mites were found by skin scraping the forehead of the 3 pups and they were considered negative for D. canis.

Litter II

Four pups of a litter of six were taken from their dam within 24 hours of their natural birth and reared in an incubator. Suspensions containing live D. canis were placed on the gauze of a commercial plastic bandage and applied to the foreheads of two pups 1 day old. Later, using the KOH method, skin samples yielded a mite from one pup 25 days post inoculation and two D. canis were recovered from the forehead of the other pup 8 weeks after inoculation.

No mites were found in skin samples from the two pups not receiving a dermal application of mites. Skin samples from the eyelid, forehead, shoulder and hip were taken at necropsy from one pup at 25 days of age and from the other at 8 weeks of age.

Two pups, also from Litter II but reared by the dam, were also negative for mites. Skin samples from the eyelid, forehead, shoulder and hip were taken at necropsy from one pup at 25 days of age. A skin sample, approximately 1 square cm, was taken by biopsy from the other pup at 10 weeks of age and no mites were found by the KOH method.

The last-mentioned pup, when 10 weeks old, was placed in a pen for 2 weeks with two pups exhibiting severe demodectic mange. Three weeks after separation from the two pups with demodectic mange, the pup developed small lesions on the forehead and lower jaw. At 4 months of age (1 month post inoculation) this pup had extensive demodectic mange. The mandibular and prescapular lymph nodes were enlarged and contained many mites.

Litter III

Shortly before delivery of six pups, a beagle bitch was examined several times by skin scrapings. No mites were found. Four of the pups were removed from fetal membranes and reared in an incubator while two pups remained with the dam. Mites were applied to the forehead of two hand-reared pups at 4 weeks of age. The other two hand-reared pups and the pups reared by the dam were not exposed to D. canis.

At 6 weeks of age, living mites were found in skin scrapings from the two pups that were inoculated with mites. No mites were found by skin scrapings or by the KOH method in the samples from forehead, shoulder, flank or hip of the other four pups.

Litter IV

No mites were found by skin scrapings from the forehead, shoulder, or hip of a mixed terrier bitch the day before delivery of seven pups. Four pups were taken from the dam 1 day after birth and reared in an incubator. The remaining three pups were reared by the dam.

Mites in skin scrapings were applied to the forehead of 1 hand-reared pup, 1 day old. At 11 weeks of age, living mites were found in skin scrapings from the forehead.

At 10 weeks of age, one hand-reared pup and one pup reared by the dam were placed in a small pen with $5\frac{1}{2}$-month-old pup exhibiting severe demodectic mange. After 3 days, they were removed from the pen. One week later, living mites were found in skin scrapings from both pups.

At 11 weeks of age, skin samples from the forehead, shoulder and flank of the four pups not exposed to mites (2 hand-reared, 3 reared by dam) were free of the parasite (KOH method).

Litter V

At whelping, the dam had mild demodectic mange. Three pups were isolated from her about 6 hours after birth and reared in an incubator. One pup died at age $2\frac{1}{2}$ days. Mites were found in histologic preparations of skin from the forehead, principally between the eyes (Fig. 2). Mites were found in skin scrapings from the remaining two pups at 3 months and in skin samples at 8 months. Neither had any visible lesions.

Litter VI

A pup, taken by Cesarean section, was reared by hand. At $3\frac{1}{2}$ months of age, the pup was placed in a pen with two dogs having low populations of D. canis but no skin lesions. No mites were recovered by the KOH method from skin samples of eyelid, nose, cheek, forehead, flank or hind leg $4\frac{1}{2}$ months later.

Litter VII

A litter of five pups was delivered naturally by a dam with a mild squamous demodectic mange. Three of the pups were isolated from the dam 3 days after birth and reared in an incubator. The other two pups remained with the dam. D. canis were found in skin scrapings from all five pups at 2 months of age. Lesions developed on three of the pups; however, only one pup developed chronic demodectic mange (lasting 2 years).

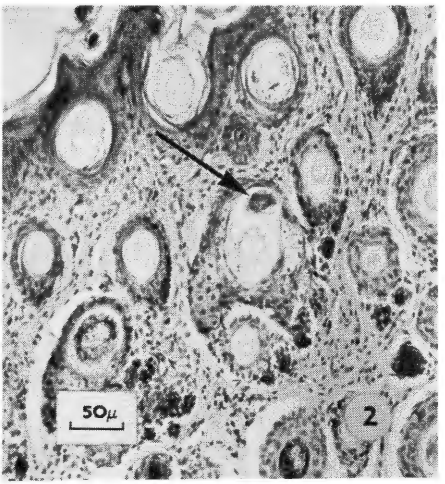

Figure 2. Demodex canis (arrow) in hair follicles of pup, $2\frac{1}{4}$ days old. Hematoxylin and eosin.

Prenatal Transmission

Although there are speculations in the literature as to the possibility of infection of fetuses by D. canis (Enigk, 1949; Kirk, 1950; Rubin, 1957), no documented evidence indicating prenatal transmission has been published to our knowledge.

Observations concerning prenatal transmission were made on two litters.

Litter VIII

A terrier of mixed ancestry, with a moderate squamous mange, delivered seven pups. Four pups were taken immediately at birth and their placental membranes removed by hand. Great care was exercised not to contaminate the pups with D. canis from the dam. Each of the four pups was placed in a separate compartment of an incubator for hand rearing. The other three pups remained with the dam.

Euthenasia of the four hand-reared pups was performed at 24, 57, 57, and 85 days of age. Skin samples from forehead, shoulder, flank and hip were carefully examined by the KOH method. All were negative for D. canis.

All three pups reared by the dam were positive for D. canis. Mites were recovered by the KOH method from forehead skin samples taken at necropsy from one pup 24 days old. No mites were recovered from its mandibular lymph nodes. The remaining two pups had clinical symptoms of demodectic mange at 57 days of age. Three weeks later these two pups were almost hairless, emaciated, edematous and muscularly weak. In addition to very high populations of D. canis, the pups were parasitized by hookworms (Ancylostoma caninum) and ascarids (Toxocara canis). At 85 days of age, one pup died and euthanasia was performed on the other. D. canis were found (by the KOH method) in mandibular, prescapular, superficial inguinal and popliteal lymph nodes from each pup. More than 100 mites, including all stages of D. canis (few ova), were recovered from four lymph nodes of one pup.

Litter IX

A boston terrier was almost hairless at whelping. Mites were recovered (KOH method) from a prescapular lymph node of this dam 2 months before whelping. Three pups were removed by hand from placental membranes at birth.

No mites were recovered by the KOH method from the entire cadavers of the two pups that were delivered dead. The remaining pup was reared in an incubator until euthanasia was performed at 3 weeks of age. No mites were found when the skin of forehead, shoulder and hip mandibular lymph nodes were examined by the KOH method.

The absence of D. canis in this litter demonstrated that a bitch with extensive skin lesions, with secondary bacterial infection in the skin, and with mites in the lymphatic system could produce pups which were free of D. canis at birth. It was unfortunate that only one pup survived and that a control pup could not be reared with the dam. However, in view of the ease with which mites were induced to establish populations on the pups of the litters previously discussed, we have no doubt that any pups reared by such a bitch would have D. canis in their skin.

Peroral Transmission

Cánepa and da Graña (1945) reported peroral transmission of mites to eight pups, 1-3½ months of age. Rubin (1957) stated: "I have been able to cause demodicosis by feeding and by intraperitoneal injection of infected material." Despite publication of these apparent successes, the per os route of transmission has failed to gain support from other researchers. Unsworth (1946) and Enigk (1949), in independent studies, presented evidence indicating that puppies could not be infected with D. canis by this method.

Unsworth attempted transmission to three puppies (age not given) by feeding them each 1 square inch of skin from an active demodectic mange lesion. Another pup was fed a prescapular lymph node (the corresponding lymph node from the opposite side of the body contained 614 mites). No mites were found in the four pups after 1 month. Skin samples and mandibular, prescapular, mesenteric, portal, and iliac lymph nodes were examined.

Enigk (1949) concluded that peroral transmission did not occur in 12 dogs ranging in age from 6 days to 18 months. Each dog was fed material containing living mites. The peroral inoculations varied from six feedings, of at least 100 mites each, during a 4-day period to 14 inoculations in 47 days. Examination techniques varied and were performed from 5 days to 10 months after the last inoculation. Some of the dogs were examined only by skin scraping. Others were examined more thoroughly. At necropsy the skin was detached from the cadaver, moistened, rolled, and allowed to decay in a closed container for 5 days. The hair was then pulled from the dermis; skin scrapings were made from the epidermis and examined. Internal organs were macerated and the sediment was examined for mites. No mites were found in samples of liver, spleen, lung, kidney, intestine and the following lymph nodes: bronchial, portal, mesenteric, mandibular, retropharyngeal, prescapular, axillary, iliac, lumbar, superficial inguinal, and popliteal.

We attempted peroral inoculation of D. canis to 3 pups of Litter X.

Litter X

Two days after birth, five pups were removed from their dam and reared in individual compartments of an incubator. Skin scrapings suspended in saline were administered per os with a pipette to three of the pups, aged 3 weeks. The saline suspension contained numerous D. canis in all stages of development. Peroral inoculation was repeated 2 days later. Subsequent examination of skin scrapings and biopsy of prescapular lymph nodes from the inoculated animals revealed no mites. After an observation period of 6 months in which no D. canis were found, the dogs were considered free of the mite.

SUMMARY

The criterion used for positive transmission of D. canis to pups was the presence of living mites in the skin rather than visible skin lesions. Transmission was accomplished by direct application of skin scraping suspensions containing D. canis to forehead skin of seven pups, 5 hours to 4 weeks old, from four litters. After exposure to the mites, these pups were isolated and reared in incubators. Mites were recovered from all seven inoculated pups, and two had well established populations of D. canis 3 months after inoculation. Nine other pups from the same four letters were also reared by hand, not exposed to D. canis, and no mites were found at examination.

Transmission was also accomplished by confining three pups, 10-11 weeks of age, in pens for 3-14 days with pups exhibiting severe demodectic mange lesions. One pup developed a severe demodectic mange, 1 month post inoculation. Pups from the same litters, reared in a similar manner but not exposed to pups with demodectic mange, remained free of mites.

Eight pups from three litters were reared by dams with demodectic mange; all became infested with D. canis. Five of the pups did not have visible skin lesions but three developed demodectic mange.

Prenatal transmission of D. canis did not occur in two litters delivered from dams with demodectic mange. Four pups that were removed from fetal membranes and reared in isolation had no mites in their skin at necropsy. Three litter-mates, reared by the dam, were positive for D. canis. The second dam was almost hairless at whelping of three pups. These pups were removed from fetal membranes and the entire bodies were macerated. No mites were found upon examination of the residue. The third pup was removed from fetal membranes and reared in isolation. Upon examination of the skin and mandibular lymph nodes at 21 days, no mites were found.

Peroral transmission was not successful when mites were fed to three pups 3 weeks old.

It was concluded that populations of D. canis could establish in the skin of young pups either after contact with diseased skin of dogs or after exposure to skin scraping suspensions containing D. canis.

Transmission of the mites to pups per os or in utero was not successful.

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KINETICS OF THE TIN(II) REDUCTION OF SOME FERRICINIUM IONS[1]

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ABSTRACT. The kinetics of the reduction of ferricinium, dimethylferricinium and ethylferricinium ions by stannous chloride have been studied. The results indicate that four chloride ions, one ferricinium ion and one stannous ion are involved in the activated complex. The ratios of the rates of reduction are 1:(3-8):17 for dimethylferricinium, ethylferricinium and ferricinium ions respectively. This probability reflects the ease of formation of the ferricinium chloride complexes.

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The reaction between ferric and stannous ions has been studied by many investigators (1-8). In particular, Duke and Pinkerton (9) studied the role of halide ions in the reaction and concluded that in the presence of chloride ion there is a first order dependence in ferric ion concentrations and, in the range 0.05 to 0.25 M, fourth order dependence on chloride is dominant. They also concluded that the possibility of reaction would be enhanced if more than three chloride ions, which they considered to be the minimum, were present in the activated complex. It is possible that in a higher range of chloride concentration the dependence on chloride may be raised above four to five or more (the role of ferric and stannous chloride complexes of higher order would become more important).

There has been some question as to the mechanism when two ions of like charge react. An anion bridge has been postulated by which an electron tunneling effect can occur (9-12). Since the shielding of iron between two aromatic organic rings occurs in ferrocene and its derivatives, a possibility arises for studying an electron tunneling effect through the rings and also the dependence of the rate on ease of formation of halide complexes with the iron. Some work on the ferricinium-stannous reaction has been done by Wilkinson and co-workers (13) but rate studies were not attempted. Duke and Peterson have made some rate studies on the ferricinium ion (14).

In this paper some derivatives of ferrocene are investigated for order in chloride ion and relative rates of reaction of the oxidized ions with stannous ion in solutions of varying chloride concentration.

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[1] Contribution No. 1383. Work was performed in the Ames Laboratory of the U.S. Atomic Energy Commission.

[2] Present address: Texas A and M University, College Station, Texas.

[3] Present address: Ethyl Corp., Baton Rouge, Louisiana.

EXPERIMENTAL

Materials:

Solutions of HCl, $HClO_4$, $SnCl_2$, and $Fe(ClO_4)_3$ were prepared in various concentrations and standardized by the usual methods. Using a standard solution of 0.1 N $Fe(ClO_4)_3$ in 2 N perchloric acid, the ferrocene derivatives, which were obtained from Dupont and Linde Air Products, were oxidized to their respective ferricinium ions.

Procedure:

Solutions of the appropriate concentrations of chloride and stannous ions were prepared in 250 ml volumetric flasks and diluted sufficiently to allow about 15 cc below the mark. They were then thermostated in a constant temperature bath at 25.2 ± 0.5°C and allowed to equilibrate. Then the ferricinium ion solution was added. The flask was quickly diluted to the mark and shaken. Samples were withdrawn and filtered in a colorimeter cell, using a sintered glass funnel, grade F, on a vacuum system. The optical density was then read on a Coleman Junior Spectrophotometer at λ = 615, using water as reference. In all cases the pH was very low to minimize both the hydrolysis of the tin and the rate acceleration by hydroxide ion.

The oxidation was done with $Fe(ClO_4)_3$ in 2 N $HClO_4$ since other agents (H_2O_2, $KClO_4$, HNO_3, $KBrO_3$, I_2, Cl_2) were found either to destroy the compound along with oxidation or were not efficient enough to give desired concentrations. Fe(II) is not known to complex with chloride, and the reduction of Fe(III) is so fast as to be unnoticed in these experiments (10). Furthermore, ferric chloride complexes do not absorb at 615 μ.

Initial experiments with dimethyl ferrocene resulted in first-order rate plots that had two distinct linear sections, particularly for intermediate and lower chloride concentrations. Since no two-step reduction mechanism could be evolved and, considering the behavior of the slopes with varying concentrations of reactants, the possibility of contamination became suspect. After usual purification procedures (sublimation, adsorption chromatography, reprecipitation, etc.) failed, an attempt was made to use the reaction being studied for purification. A solution containing 2.7 M (Cl^-), 0.17 M (Sn^{++}), and the dimethyl ferricinium ions, was allowed to react for 50 min. and then filtered. The filtrate was allowed to finish reacting and then the precipitate of dimethyl ferrocene recovered. Rough estimation indicated an impurity of some 15% had been reduced to about 2-4%. Determinations using this purified material had little or none of the two-step character. The melting point of the sample was raised 5 degrees to 42°C.

RESULTS AND DISCUSSION

Since high tin and chloride concentrations were used, corrections were made for the three stannous chloride complexes, reported by Vanderzee and Rhodes (16) to have measurable equilibrium constants ($SnCl$, B_1 = 11.6; $SnCl_2$, B_2 = 52; and $SnCl_3^-$, B_3 = 33). Furthermore, since the concentration of oxidant was very much lower, the stannous

or which occurs as trace ions in the absence of added salt. Previous results, which state that in the presence of salt the value of M^{ext} should be $MPX_Z - (Z_p/2)MBX$, are shown to arise from assumptions or definitions of the polymer unit. In addition, it is concluded that in a polar solvent the value of M^{ext} is never equal to $MPX_Z/(1 + Z_p)$ in the absence of salt because of the presence of trace salts or the presence of ions from the dissociating solvent molecules.

III. HETEROGENEITY

Introduction

In Part I (13) general equations were developed for the determination of the molecular weight of a charged polymer PX_Z by the sedimentation-equilibrium method. The equations developed were applied to a homogeneous polyelectrolyte. In this part the equations are applied to a heterogeneous polyelectrolyte.

Previously, the method of Johnson et al. (24) where the polymer PX_Z is defined as $[PX_Z - (Z/2)BX]$, has been modified and applied to a heterogeneous system (10). Here BX is a low-molecular weight electrolyte and Z_P is the electrostatic charge of the dissociated polymer PX_Z. However, in the development (10) of the concentration coefficient B for this $[PX_Z - (Z/2)BX]$ component, interactions between different polymer species and between polymer and salt were neglected. In this part these coefficients and cross-coefficients will be considered. The equations developed previously (10) for the evaluation of $\bar{M}_{W,r}$, $\bar{M}_W$, $\bar{M}_{Z,r}$ and $\bar{M}_Z$ will be applied to the PX_Z component. In addition, equations for the number-average molecular weights $\bar{M}_{n,r}$ and $\bar{M}_n$ will be developed.

Determination of the B Coefficient

General equations. Consider a system that contains a solvent, a low-molecular weight electrolyte BX and a heterogeneous polymer $P_iX_{Z_i}$, which completely dissociates into the components $P_i^{+Z_i} + Z_iX^-$. In Part I it was shown that at the radius r the expression for the $P_iX_{Z_i}$ component for ultracentrifugal equilibrium is:

$$2A_i\, r\, dr = \sum_{\substack{j=1 \\ j\neq BX}}^{q} \left(\frac{\partial \ln m_i}{\partial m_j}\right)_{T,p,m_k} dm_j + \sum_{\substack{j=1 \\ j\neq BX}}^{q} \left(\frac{\partial \ln \gamma_{N,i}}{\partial m_j}\right)_{T,p,m_k} dm_j + \left(\frac{\partial \ln m_i}{\partial m_{BX}}\right)_{T,p,m_k} dm_{BX} + \left(\frac{\partial \ln \gamma_{N,i}}{\partial m_{BX}}\right)_{T,p,m_k} dm_{BX} + \left(\frac{1}{RT}\right) dW_i \qquad (1)$$

where $A_i \frac{M_i(1-\bar{V}_i\rho)\omega^2}{2RT}$, where $i = P_iX_{Z_i}$ and where

$$dW_i = RT \sum_{j=1}^{q+1} \left(\frac{\partial \ln \gamma_{E,i}}{\partial m_j}\right)_{T,p,m_k} dm_j + RT \sum_{j=1}^{q+1} \left(\frac{\partial \ln \gamma_{d,i}}{\partial m_j}\right)_{T,p,m_k} dm_j$$

The nonelectrostatic interaction is manifested in the $\gamma_{N,i}$ term while the electrostatic interaction ($\gamma_{E,i}$) and dipole-dipole interaction ($\gamma_{d,i}$) is manifested in the dW_i term. If binding or complexing between polymer units is absent, then the term $\sum^{q} \left(\partial \ln m_i/\partial m_j\right)_{T,p,m_k} dm_j$ in equation 1 becomes $d \ln m_i$. Similarly, if binding between the salt BX and the polymer $P_i^{+Z_i}$

striking. Equation 4.9 therefore readily explains the results of Johnson et al. (26).

The true value of (M_D/P) or its equivalent $(M_{PX_Z} - Z_P M_{BX})/Z_P$ may actually be approximately the same as the true value of $M_{PX_Z}/(1 + Z_P)$. That is, the factor $-Z_P M_{BX}$ may tend to decrease the value of M_D/Z_P to the same extent that the factor $(1 + Z_P)$ instead of Z_P tends to decrease the value of $M_{PX_Z}/(1 + Z_P)$. Thus by using $(1 + Z_P)$ instead of Z_P the difference between M_{PX_Z} and $(M_{PX_Z} - Z_P M_{BX})$ may be compensated. This factor would explain why Johnson et al. (26) obtained approximately the correct value for the electrostatic charge Z_P.

At extremely low polymer concentrations the factor $(1/Z_P)$ of equation 4.5 will become significant. Equation 4.5 shows that the apparent molecular weight will change from its essentially constant value to a greater value as C_P approaches zero. That is, the approximate equation 4.9 predicts a constant M^{app} over relatively high values of C_P. As C_P is lowered $(1/Z_P)$ in equation 4.5 becomes more significant resulting in a change in this constant value. Ultimately the apparent molecular weight will become equal to $M_{D,r}$ when C_P equals zero. This conclusion agrees with the results of Kronman and Timasheff (32) who found by turbidity measurements that at low concentrations the apparent molecular weight increased from a relatively constant value. The results of Johnson et al. (26) were obtained from concentrations much higher than those of Kronman and Timasheff (32). Consequently, this increase was not observed by Johnson et al. (26) except for a scatter at their lower concentrations. Thus it is concluded that the data of Johnson et al. (26) does not refute the conclusion that the extrapolated value $(M_{PX_Z} - Z_P M_{BX})$ instead of $M_{PX_Z}/(1 + Z_P)$ is obtained in the absence of added salt. Rather, if anything, their (26) data together with that of Kronman and Timasheff (32) support the conclusions made in this paper.

The results of Casassa and Eisenberg (3) were not included in the above discussion since their approach can be considered as a modification of the component $[PX_Z - (Z/2)BX]$ as used by Johnson et al. (24). Casassa and Eisenberg's (3) component equals the component of Johnson et al. (24), when a univalent salt BX is considered and when $\beta_{23} = (\partial \ln \gamma_2/\partial m_3)_{T,p,m_2} = (\partial \ln \gamma_{PX_Z}/\partial m_{BX})_{T,p,m_{PX_Z}} = 0$. Casassa and Eisenberg (3) define their component as $A^* = [A_{PX_Z} - A_{BX}(a_{23}/a_{33})]$ where $a_{23} = (\partial \ln a_{PX_Z}/\partial m_{BX})$ and $a_{33} = (\partial \ln a_{BX}/\partial m_{BX})$. Hence, the value of their extrapolated molecular weight is related to a definition of a fictitious component just as in the case of the extrapolated value given by Johnson et al. (24). The discussion given above concerning the equations of Johnson et al. (24) therefore applies to the equations of Casassa and Eisenberg (3). Hence, the above conclusions concerning the extrapolated molecular weight remain the same.

Synopsis

A review of the literature showed that the extrapolated molecular weight of a polymer (M^{ext}) is related to the true molecular weight M_{PX_Z} by the relationship $M^{ext} = M_{PX_Z} - Z_P M_{BX}$ for a polymer having a net charge Z_P and for the condition $V_B = V_P$. Here M_{BX} is the molecular weight of a supporting electrolyte which is either added to the solution

$$\mu_{BX,\alpha} - \left(\frac{M_{BX}\omega^2 r^2}{2}\right)_\alpha + W_{BX,\alpha} = \mu_{BX,\beta} - \left(\frac{M_{BX}\omega^2 r^2}{2}\right)_\beta + W_{BX,\beta} \tag{4.70}$$

If we now assume as in developing equation 4.5 that the electrostatic potential term does not contribute significantly to the total potential and, in addition, that the sedimentation force on the low molecular weight electrolyte is also negligible, then equation 4.70 becomes:

$$\mu_{BX,\alpha} = \mu_{BX,\beta} \tag{4.71}$$

The definition of the chemical potential μ_{BX} is

$$\mu_{BX,} = \mu^{\circ}_{BX} + RT \ln m_B + RT \ln m_X \tag{4.72}$$

assuming that the activity coefficients $\gamma_{N,B}$ and $\gamma_{N,X}$ are unity.

Substitution of equation 4.72 into 4.71 gives the Donnan equilibrium relationship:

$$\frac{m_{B,\alpha}}{m_{B,\beta}} = \frac{m_{X,\beta}}{m_{X,\alpha}} \tag{4.73}$$

At equilibrium Y moles of B^+ and Y moles of X^- will have moved from $r = \beta$ to $r = a$. The concentration of $m_{X,a}$ and $m_{X,\beta}$ will then be $m_{X,a} = m_{B\overset{\circ}{X}} + Z_p m_{p,a} + Y$ and $m_{X,\beta} = m_{B\overset{\circ}{X}} + Z_p m_{P,B} - Y$ where $m_{B\overset{\circ}{X}}$ is the initial concentration (before centrifugation) of the salt BX. However, if the salt is due only to the presence of extraneous matter, then both $m_{B\overset{\circ}{X}}$ and Y will be negligible in comparison to $Z_p m_p$. Consequently, the values $m_{X,a}$ and $m_{X,\beta}$ are $m_{X,a} = Z_p m_{p,a}$ and $m_{X,\beta} = Z_p m_{P,\beta}$ assuming that Z_p is the same at all radii. Since the distribution of a polymer in the ultracentrifugal field is actually a function of its activity, then it can be assumed that $m_{X,a}$ and $m_{X,\beta}$ are proportional to the respective polymer activities instead of the polymer concentrations. Hence, equation 4.73 becomes:

$$\frac{m_{B,\alpha}}{m_{B,\beta}} \equiv \frac{c_{B,\alpha}}{c_{B,\beta}} \equiv \frac{\bar{a}_{P,\beta}}{\bar{a}_{P,\alpha}} \tag{4.74}$$

Substitution of the relationship given in equation 4.74 into equation 4.6 gives:

$$\left[\frac{(1-\bar{V}_P\rho)\omega^2}{2RT}\right]\left(\frac{M_D}{Z_P}\right) d(r^2) = d \ln \bar{a}_P \tag{4.8}$$

This approximation is true only when $C_P >> C_B$ and when C_P is relatively large. Integration of equation 4.8 yields:

$$\ln\left(\frac{m_{P,\beta}}{m_{P,\alpha}}\right) + \ln\left(\frac{\bar{\gamma}_{P,\beta}}{\bar{\gamma}_{P,\alpha}}\right) = \left(r_\beta^2 - r_\alpha^2\right)\left(\frac{M_D}{Z_P}\right)\left(\frac{1-\bar{V}_P\rho}{2RT}\right)\omega^2 \tag{4.9}$$

since $\bar{a}_P$ equals $m_P\bar{\gamma}_P$. Equation 1a of reference 26 is:

$$\ln\left(\frac{m_{P,\beta}}{m_{P,\alpha}}\right) + \ln\left(\frac{\bar{\gamma}_{\pm\beta}}{\bar{\gamma}_{\pm\alpha}}\right) = \left(r_\beta^2 - r_\alpha^2\right)\left(\frac{M_{PX_Z}}{1+Z_P}\right)\left(\frac{\omega^2}{2RT}\right) - \left(\frac{M_{PX_Z}}{1+Z_P}\right)\left(\frac{\omega^2}{2RT}\right)\int_{P_\alpha}^{P_\beta} \bar{V}_\beta \rho \, r\, dr$$

In equation 4.9 the term (M_D/Z_P) occurs instead of $M_{PX_Z}/1 + Z_P)$. In addition, the partial volume has not been corrected in equation 4.9, i.e., the integral occurring in equation 1a of reference 26 is omitted in equation 4.9. Nevertheless, the similarity between the two equations is

The above relationship given in equation 4.1 is interesting since as pointed out by Lamm (33) it does not involve the concentration of the polymer or that of the buffer. In other words, the polymer cannot be thermodynamically treated as an isolated component in a one-phase system by neglecting the presence of trace ions due to the dissociation of the solvent or other factors (see Introduction). Furthermore, the relationship holds even though there is negligible interaction between polymer molecules, i.e., absence of concentration dependence. Hence, it must be concluded that the molecular weight obtained by extrapolating to zero polymer concentration in the absence of salt is not $M_{px_z}/(1 + Z_p)$ as previously thought (26, 34). Rather, it is $M^{ext} = M_{px_z} - Z_p M_{BX}$ for $\bar{V}_{BX} = \bar{V}_{px_z}$ because a complete absence of other ions can never be attained in a polar solvent.

The experimental work of Johnson et al. (26) appears to contradict the statement that $M_{px_z}/(1 + Z_p)$ (or their (26) equivalent expression $M_{px_z}/\sqrt{}$) is not the extrapolated molecular weight in a polar solvent. They obtained their equations by assuming that the polyelectrolyte can be treated as given in equation 1 with slight modifications in order to account for changes in pressure and density. (These factors may be important for extremely low molecular weight polyelectrolytes but can be neglected in most cases (55).) That is, the equations of Young et al. (58) were applied by Johnson et al. (26) to a polyelectrolyte without considering the common ion effect. It will now be shown that their (26) experimental results do not contradict the statement that $M_{px_z}/(1 + Z_p)$ is never the extrapolated molecular weight.

Equation 4.0 can be put into the following form:

$$M_{D,r} = M^{app}_{P_{I,r}} \left[1 + B_{D,r} M_{D,r} C_{P,r}\right] \tag{4.4}$$

If we now assume that $d(Z_p/M_D) = d \ln \gamma_{N,P} = dW_D = 0$, then the only term left in the $B_{D,r}$ coefficient of equation 4.2 is $(Z_p/M_D)_r(-d \ln C_B/dC_p)_r$. These assumptions may be valid since silicotungstic acid contains no titratable groups in the pH range studied (26) and, hence, the value of Z_p would not be expected to vary. In addition, zwitterions are absent and thus the electrostatic term dW is most likely negligible. Substituting into equation 4.4 the $(d \ln C_B)$ term for the $B_{D,r}$ coefficient and the value of $M^{app}_{P,r}$ as given in equation 4.3 and multiplying both sides of equation 4.4 by $[d(r^2)/Z_p]$ gives:

$$\left[\frac{(1-\bar{V}_p\rho)\omega^2}{2RT}\right]\left(\frac{M_D}{Z_p}\right) d(r^2) = d \ln C_p \left[\left(\frac{1}{Z_p}\right) + \left(\frac{-d \ln C_B}{d \ln C_p}\right)\right] \tag{4.5}$$

If the quantity $(1/Z_p)$ is much less than the quantity $(-d \ln C_B/d \ln C_p)$, then equation 4.5 can be approximated as:

$$\left[\frac{(1-\bar{V}_p\rho)\omega^2}{2RT}\right]\left(\frac{M_D}{Z_p}\right) d(r^2) = -d \ln C_B \tag{4.6}$$

In order to evaluate fully the $-d \ln C_B$ term under the experimental conditions used by Johnson et al. (26), let us again examine the basic equation given for ultracentrifugal equilibrium for the salt BX between the radii $r = \alpha$ and $r = \beta$ (see equation 2.4 of Part I):

$dm_B = dm_x$ is not valid, it follows that the approximation d ln m_B = d ln m_x will also be in error.

Lamm's (33) approximation may be summarized by stating that the change in $(m_x - Z_p m_p)$ with cell radius equals the change in m_x with cell radius when the value of m_x is much larger than that of $Z_p m_p$, e.g., when m_p is extrapolated to low values. The author concludes that this approximation leads to erroneous results with regard to the extrapolated molecular weight. It must be emphasized that Lamm's (33) equation (eq. 7.1) was obtained from the same basic equations used in deriving the contradictory result given in equation 4.1.

Discussion

Equations 4.1, 5.5, 6.5 and 7.1 show that at infinite polymer dilution the extrapolated molecular weight obtained for a charged polymer by means of equilibrium ultracentrifugation is not the true molecular weight. However, a close examination of these theoretical values for the extrapolated molecular weight M^{ext}, i.e., M_D, M_2, M* and M**, at the radius r for a homogeneous polymer shows there are variations in the relationships between the extrapolated value to the true value of the molecular weight. The question arises as to which result is correct. The value of (M^{ext}/M_{px_z}) is $[1 - Z_p(M_{BX}/M_{px_z})]$ for equation 4.1, $[1-(Z_p/2)(M_{BX}/M_{px_z})]$ for equations 5.5 and 7.1, and $[1-Z_p(M_{BX}/M_{px_z})+ (Z_p/2)(1 + Z_p)(M_{BX}/M_{px_z})^2]$ for equation 6.5 assuming that $Q=(1-\bar{V}_B\rho)/(1-\bar{V}_P\rho) = 1$, $(\theta_B/\theta_P)=1$ and $C_p = 0$.

Inspection of equation 6.5 shows that it is essentially equal to equation 4.1. The slight variation resulting when $Q \neq 1$ but $(\theta_B/\theta_p) = 1$ is most likely due to approximations made in deriving equation 6.5. That is, $(Q + 1)/2$ results from equation 6.51 instead of Q as from equation 4.1 for $(\theta_B/\theta_p) = 1$. The direct derivation of Williams et al. (55) which was derived assuming that dW = d Z_p = 0, can also be shown to yield equation 4.1 for the extrapolated molecular weight (see their (55) equations 54 and 55). In addition, Lamm's results (33) are due to approximations (see development of equation 7.0). Hence, in all cases except the derivation for the $[PX_z - (Z/2) BX]$ component (equation 5.4), the extrapolated molecular weight is equal to $M_p[1 - (Z_p)(M_{BX}/M_{px_z})Q]$ unless assumptions are made in the thermodynamic relationships. In addition as shown, the value of (M^{ext}/M_{px_z}) as obtained in equation 5.5 actually results from the definition of the $[PX_z - (Z/2)BX]$ component in the $B_{2,r}$ coefficient. It is therefore concluded that the definition given in equation 4.1 gives the true relationship between extrapolated and true molecular weights of the polymer.

In all equations binding between salt and polymer was not considered. Experimentally, the association of ions around a polyelectrolyte appears to reduce the effect of the electrostatic charge Z_p. (12) It will be shown in Part V that M^{ext} is $M_{PX_z}[1 - (Z_p-\Gamma)(M_{BX}/M_{px_z})Q]$ when binding is considered. Here Γ is the binding coefficient as defined by Williams et al. (55). Experimental results on bovine plasma albumin suggest that $\Gamma = Z_p$. (12) This type of association can be considered as being similar to the preferential association of one type of solvent molecule over another by a polymer in a binary solvent system.

Lamm (33) also observed that similar results could be obtained if equation 3.1 is approximated as:

$$\frac{d m_X}{m_X} + \frac{d m_B}{m_X} = 2A_{BX}(r\,d\,r) \qquad (7.3)$$

That is, let $m_X = m_B$ for the electroneutrality equation. This approximation by itself and not as applied to eq. 7.3 is essentially correct for a low concentration of polyelectrolyte. However, the point that must be emphasized and which invalidates this approximation is that d ln m_X or (dm_X/m_X) and not dm_X is eliminated from the equation for PX_Z (equation 3.0). In other words, a derivative of m_B is involved in the expression d ln m_B. An approximation of m_B therefore involves an approximation of a derivative and not of a numerical expression.

Consider, for example, the application of the electroneutrality expression ($m_X = m_B + Z_p m_p$) to equation 3.1 when dW = 0:

$$d \ln m_X + d \ln (m_X - Z_P m_P) = 2A_{BX}\, r\, d\, r \qquad (7.4)$$

In equation 7.4 the definition of the term d ln $(m_X - Z_p m_p)$ is

$$\lim_{\Delta r \to 0} \ln \left[(m_X - Z_P m_P)_2 / (m_X - Z_P m_P)_1 \right]$$

The ratio in this logarithmic term can be influenced tremendously by the changes occurring in $Z_p m_p$ with cell radius even though the order of magnitude of m_X may be much larger than that of $Z_p m_p$. One must also recollect that extrapolation to "zero" polymer concentration does not mean that the concentration of the polymer is zero but rather that it is infinitely dilute. If m_p were zero, then only the properties of the salt BX would be obtained. Hence, equation 7.4 can not be approximated as equation 7.3 since d ln m_X is used. As would be expected on the basis that d ln m_X and not dm_X is eliminated, Lamm (33) observed that the approximation d ln m_X = d ln m_B gave the same result as that obtained from the approximation $m_X = m_B$.

Lamm's (33) approximation may also be considered from a different approach. Again consider the equation $m_X = m_B + Z_p m_p$. At infinite dilution of PX_Z this equation becomes essentially $m_X = m_B$. Taking the logarithm of both sides and letting molalities equal molarities, then $m_X = m_B$ becomes $\ln(C_B/M_B) = \ln(C_X/M_X)$. Differentiation of this result gives d ln C_B = d ln C_X which is the approximation used by Lamm (33). Equation 3.1 then becomes 2 d ln $C_X = 2A_{BX}(r\,d\,r)$ and, hence, d ln C_X may be eliminated from equation 3.0 giving a resulting equation having the term $M_{PX_Z} - (Z/2)\, M_{BX}$. The error in differentiating the logarithm term was discussed above. The error involved in differentiating an approximation can also be considered by examining the differential of original approximation $m_X = m_B$, i.e., $dm_X = dm_B$. Differentiating the original equation gives $dm_X = dm_B + Z_p dm_p$ assuming Z_p is constant. While m_X may be essentially equal to m_B at low values of m_p, the value of dm_X may be quite different from dm_B since the magnitude of $Z_p dm_p$ can now approach or exceed that of dm_B. In addition, dm_B should approach zero as m_p approaches zero since dm_B is a function of m_p. Hence, the approximation $dm_B = dm_X$ will be invalid. This approximation may be restated as $dm_B = dm_X = d(m_X - Z_p m_p)$. As in the ratio term of the logarithmatic expression, a change in $(m_X - Z_p m_p)$ with cell radius is approximated as a change in m_X. Thus, if the approximation

Q and (θ_B/θ_p) equal 1, then equation 6.51 becomes:

$$M_r^* = M_{PX_Z}\left\{1-Z_p\left(\frac{M_{BX}}{M_{PX_Z}}\right)\right\} \tag{6.52}$$

Thus, the same relationship is obtained for the extrapolated molecular weight as given in the direct derivation (equation 4.1).

A similar result is obtained by examining equation 63 of reference 55. That is, equation 6.52 results from equation 63 of reference 55 if $Q = (\theta_B/\theta_p) = 1$ and if it is assumed that the squared term $[(Z_p/2)(M_{BX}/M_{px_z})]^2$ is less than $Z_p (M_{BX}/M_{px_z})$. Since the quantity $(Z_p/2)(M_{BX}/M_{px_z})$ is under normal circumstances less than 1, then this approximation is valid. One might predict that introduction of a third physical factor G' would yield the result:

$$M_r^* = M_{PX_Z}\left\{1-Z_p\left(\frac{M_{BX}}{M_{PX_Z}}\right)\left[\frac{Q+G'+(\theta_B/\theta_P)}{3}\right]\right\} \tag{6.53}$$

That is, the third physical factor G' would be similar to the physical factors Q and (θ_B/θ_p) of equation 6.51.

Lamm's (33) Approximation. In order to obtain Lamm's (33) equations in the terminology given above, some of its derivations will be summarized. It will also be shown that his assumptions lead to erroneous results with regard to the value of the extrapolated molecular weight. Lamm (33) used the basic equations 2.0, 2.1, 3.0 and 2.1 if it is assumed that d Z = 0, that dW = 0 and that the activity coefficients are constant, i.e., $a_x = m_x$, $a_p = m_p$ and $a_B = m_B$. The concentration m_x was then obtained from electroneutrality relationship $m_x = Z_p m_p + m_B$. The derivative d ln m_x then equals d ln $[Z_p m_p + m_B]$ = d ln m_B $[1+(Z_p m_p/m_B)]$. Assuming that $[1 + (m_p/m_B)]^{Z_p} = 1 + Z_p(m_p/m_B) + Z_p(Z_p-1)(m_p/m_B)^2/2! + \ldots = [1 + Z_p (m_p/m_B)]$, then d ln m_B $[1 + (Z_p m_p/m_B)]$ = d ln m_B + Z_p d ln $[1 + (m_p/m_B)]$. In addition, Lamm (33) assumed that Z_p d ln $[1 + (m_p/m_B)] \cong Z_p$ d (m_p/m_B), i.e., $_e(m_p/m_B) = 1 + (m_p/m_B)$, and that Z_pd $(m_p/m_B) = Z_p(m_B$ d $m_p - m_p$ d $m_B)/m_B^2 = Z_p$ d m_p/m_B. These assumptions are valid (33) when $Z_p(C_p/M_p)$ is much smaller than (C_B/M_B) and when C_p d $C_B \ll C_B$ d C_p for $m_B = C_B/M_B$ $m_p = C_p/M_p$. Substitution of [d ln m_B + Z_p(d m_p/m_B)] for d ln m_x = d ln a_x into equations 3.0 and 3.1, and combination of the two equations to eliminate the d ln m_B terms gives in terms of molecular weights:

$$\frac{1}{M_r^{app}} = \frac{1}{M_r^{**}} + B_r^{**} C_{p,r} \tag{7.0}$$

where M_r^{app} is defined as $M_{p,r}^{app}$ in equation 4.3 and where

$$M_r^{**} = M_{PX_Z}\left[1-\left(\frac{Z_p}{2}\right)\left(\frac{M_{BX}}{M_{PX_Z}}\right)\left(\frac{1-\bar{V}_B\rho}{1-\bar{V}_p\rho}\right)\right] \tag{7.1}$$

and

$$B_r^{**} = \left(\frac{1}{2}\right)\left(\frac{Z_p^2}{M_P M_r^{**}}\right)\left(\frac{M_B}{C_{B,r}}\right) \tag{7.2}$$

$$\left(\frac{dn}{dr}\right)_r = \frac{2\theta_p C_{pr} A_p \left\{1-\left(\frac{Z_p}{2}\right)\left(\frac{M_{BX}}{M_{PX_Z}}\right)\left[Q+\left(\frac{\theta_B}{\theta_p}\right)-\left(\frac{C_p}{C_B}\right)-(1+Z_p)\left(\frac{M_{BX}}{M_{PX_Z}}\right)\frac{\theta_B}{\theta_p}Q\right]\right\}}{\left[1+\left(\frac{Z_p(1+Z_p)}{2}\right)\left(\frac{M_{BX}}{M_{PX_Z}}\right)\left(\frac{C_p}{C_B}\right)\right]} \qquad (6.3)$$

if it is assumed that the quantity

$$\left[\theta_B(r) C_B A_{BX}\right] \Big/ \left[1+\left(\frac{Z_p(1+Z_p)}{2}\right)\left(\frac{M_{BX}}{M_{PX_Z}}\right)\left(\frac{C_p}{C_B}\right)\right]$$

denotes the solvent $(dn/dr)_o$ in addition to the assumption that d ln g_p = d Z = 0. Thus, the molecular weight of a homogeneous polymer at the specific radius r is:

$$\frac{1}{M^{app}_{PX_Z\,r}} = \left[\frac{(1-\bar{V}_p\rho)\omega^2 r}{RT}\right]\frac{C_{p,r}\,\theta_p}{\left[\left(\frac{dn}{dr}\right)-\left(\frac{dn}{dr}\right)_o\right]_r} = \frac{1}{\bar{M}_r^*} + B_r^* C_{p,r} \qquad (6.4)$$

where

$$M_r^* = M_{PX_Z}\left[1-\left(Z_p\right)\left(\frac{M_{BX}}{M_{PX_Z}}\right)L_r\right] \qquad (6.5)$$

$$B_r^* = \left(\frac{Z_p(1+Z_p)}{2\,M_r^* M_{PX_Z}}\right)\left(\frac{M_{BX}}{C_{B,r}}\right) \qquad (6.6)$$

and

$$L_r = \left(\frac{1}{2}\right)\left[Q+\left(\frac{\theta_B}{\theta_p}\right)\right]-\left(\frac{1}{2}\right)\left[\left(\frac{C_p}{C_B}\right)_r+(1+Z_p)\left(\frac{M_{BX}}{M_{PX_Z}}\right)\left(\frac{\theta_B}{\theta_p}\right)Q\right] \qquad (6.7)$$

Here $Q=(1-\bar{V}_B\rho)/(1-\bar{V}_{PX_Z}\rho)$ and $C_{p,\,r}$ is the concentration of the polymer.

In equation 6.7 the value of (C_p/C_B) will be zero at zero polymer concentration. In addition, the term $(1 + Z_p)(M_{BX}/M_{px_z})(\theta_B/\theta_p)Q$ will almost always be much less than Q or (θ_B/θ_p). For example, M_{BX} is usually about 100 g/mole. Hence, for a polymer having a molecular weight of 10,000 g/mole the value of Z_p would have to be $Z_p = 99$ in order to make $(1 + Z_p)(M_{BX}/M_{px_z})$ equal to 1. This large ratio of charge to mass of 1/100 would be most unlikely. In addition, Q is usually less than 1 and the value of M_{BX} may be much lower than $M_{BX} = 100$. Thus, for the extrapolated molecular weight, the two terms can be eliminated from equation 6.7. Equation 6.5 then becomes:

$$M_r^* = M_{PX_Z}\left\{1-Z_p\left(\frac{M_{BX}}{M_{PX_Z}}\right)\left[\frac{Q+(\theta_B/\theta_p)}{2}\right]\right\} \qquad (6.51)$$

Thus, the net result of the introduction of the refractive indices for concentration in the ultracentrifuge equations is to incorporate any variation from unity that Q or (θ_B/θ_p) may impose. That is, Q and (θ_B/θ_p) represent the deviations from one or unity and not the deviation from zero. Only in the remote case where $Q>>(\theta_B/\theta_p)$ or $(\theta_B/\theta_p)>>Q$ will the $(Z/2)$ term occur. If the refractive indices are not substituted for concentration, then it is assumed automatically that $(\theta_B/\theta_p) = 1$. When both

Here $k_{2,r} = (\frac{1}{2})\left\{\ln[1+f] + (1/[1+(1/f)])\right\}$ where $f = (Z_p/M_2)(M_B C_2/C_B)$. Also dW_2 is defined as given in equation 5.2 and in addition

$$M_{2,r} = M_{PX_Z}\left[1-\left(\frac{Z_{P,r}}{2}\right)\left(\frac{M_{BX}}{M_{PX_Z}}\right)\left(\frac{1-\bar{V}_{BX}\rho}{1-\bar{V}_{PX_Z}\rho}\right)\right] \tag{5.5}$$

The value of $M_{2,r}^{app}$ is defined in the same manner as $M_{p,r}^{app}$ in equation 4.3. The term $\gamma_{N,2}$ is the activity coefficient products as defined by Johnson et al. (24).

Because the salt BX is incorporated into the definition of the activity $a_{2,r}$, the resulting molecular weight $M_{2,r}$ is a fictitious quantity. Hence, a factor of $(Z_{p,r}/2)$ occurs in the definition of $M_{2,r}$ (see equation 5.5) instead of the factor $(Z_{p,r})$ as obtained in the definition of $M_{D,r}$ (see equation 4.1). This $(Z_{p,r}/2)$ term can be changed by redefining the component. For example, instead of multiplying equation 5.1 by $(Z_{p,r}/2)$, one could multiply by any constant β. The activity of the resulting component can then be defined as $a_\beta = a_{PX_Z}^{\beta} a_{BX}$. Here the extrapolated molecular weight will be $M_{BX,r} = M_{PX_Z}[1-(\beta)(M_{BX}/M_{PX_Z})Q]$ where $Q=(1-\bar{V}_B\rho)/(1-\bar{V}_P\rho)$. Hence, the true relationship between M_{PX_Z} and the extrapolated molecular weight is obtained when the activity of the polymer is not redefined.

Williams, Van Holde, Baldwin and Fujita's (55) Concentrate Coefficient. Because various assumptions were made by Williams et al. (55), in deriving their extrapolated molecular weight, the effect of minimizing the number of assumptions in their derivation will be examined. Equations 2.0, 2.1, 3.0 and 3.1 are also applicable to their derivation. In addition, Williams et al. (55) relate the total refractive index gradient to the concentration gradients as follows:

$$\frac{dn}{dr} = \theta_P\left(\frac{dC_P}{dr}\right) + \theta_B\left(\frac{dC_B}{dr}\right) \tag{6.0}$$

where $\theta_P=(\partial n/\partial C_P)_B$ and $\theta_B=(\partial n/\partial C_B)_P$. By assuming that the charge Z_p and all of the activity coefficients are constant and in addition that the electrical potential term is zero, their (55) resulting equations 57 and 58 can be written as:

$$\left(\frac{dC_P}{dr}\right) = \frac{\left[Z_P(A_{BX}/Q)(C_P^2/C_B)r\right] + 2A_{PX_Z}C_P r - Z_P A_{BX} C_P r}{\left[1+\left(\frac{Z_P(1+Z_P)}{2}\right)\left(\frac{M_{BX}}{M_{PX_Z}}\right)\left(\frac{C_P}{C_B}\right)\right]} \tag{6.1}$$

and

$$\left(\frac{dC_B}{dr}\right) = \frac{A_{BX}C_B r + Z_P(1+Z_P)A_{BX}(M_{BX}/M_{PX_Z})C_P r - Z_P(A_{BX}/Q)C_P r}{\left[1+\left(\frac{Z_P(1+Z_P)}{2}\right)\left(\frac{M_{BX}}{M_{PX_Z}}\right)\left(\frac{C_P}{C_B}\right)\right]} \tag{6.2}$$

where $Q=(1-\bar{V}_B\rho)/(1-\bar{V}_P\rho)$.

Substitution of these values for $(d\,C_p/dr)$ and $(d\,C_B/dr)$ into equation 6.0 gives:

In addition, the value of $M^{app}_{P,r}$ is

$$M^{app}_{P,r} = \left(\frac{1}{C_{P,r}}\right)\left(\frac{dC_{P,r}}{r\,dr}\right)\left[\frac{RT}{(1-\bar{V}_{PX_Z}\rho)\omega^2}\right] \qquad (4.3)$$

Erlander's Modification (10) of the Johnson, Kraus and Scatchard (24) Method for Determining Concentration Coefficient B. In this modified method the polymer is defined as $(PX_Z - [\frac{Z}{2}]\,BX)$ instead of PX_Z. The values P, Z_p, X and B are defined by equations 2.0 and 2.1. In order to differentiate this definition from the PX_Z polymer definition, the subscript 2 instead of the subscript D will be added.

Equations 3.0 and 3.1 can be written:

$$d\ln a_{PX_Z} + \left(\frac{1}{RT}\right)(dW)_{PX_Z} = 2A_{PX_Z}\,r\,dr \qquad (5.0)$$

and

$$d\ln a_{BX} + \left(\frac{1}{RT}\right)(dW)_{BX} = 2A_{BX}\,r\,dr \qquad (5.1)$$

If we multiply equation 5.1 by $(Z_p/2)$ and subtract the result from equation 5.0, then the term $d\ln a_2 = d\ln a_{PX_Z} - (Z_p/2)\,d\ln a_{BX}$ of equation 1 of reference 10 is obtained. This quantity is equal to $d\ln a_{PX_Z}\,a_{BX}^{-(Z_p/2)}$ which in turn is equal to $d\ln m_p m_X^{Z_p} m_B^{-(Z_p/2)} \gamma_{N,2}^{-(Z_p/2)} = d\ln m_p m_X^{(Z_p/2)} m_B^{-(Z_p/2)} \gamma_{N,2}^{-(Z_p/2)}$

The net result of this subtraction is:

$$d\ln a_2 + \left(\frac{1}{RT}\right)(dW_2) = A_2\,d(r^2) \qquad (5.2)$$

where

$$dW_2 = dW_{PX_Z} - (Z_p/2)\,dW_{BX}$$

and where

$$A_2 = A_{PX} - \left(\frac{Z_p}{2}\right)A_{BX}$$

The only difference between this treatment and that given in the derivation of equation 4.0 is the multiplication of equation 3.1 by either $(Z_p/2)$ in order to obtain the component of Johnson et al. (24), or Z_p in order to eliminate the term $d\ln m_X$. In terms of molecular weights the expression becomes:

$$\frac{1}{M^{app}_{2,r}} = \frac{1}{M_{2,r}} + B_{2,r}\,C_{2,r} \qquad (5.3)$$

where

$$B_{2,r} = \frac{\left(\frac{1}{2}\right)\left(\frac{Z_p}{M_2}\right)^2_r\left[1-\left(\frac{d\ln C_B}{d\ln C_2}\right)_r\right]}{\left[\left(\frac{C_B}{M_B}\right)+\left(\frac{Z_p}{M_2}\right)C_2\right]} + \left(\frac{d\ln\gamma_{N,2}}{M_2 dC_2}\right)_r + \left(\frac{1}{RT}\right)\left(\frac{dW_2}{M_2 dC_p}\right)_r + k_{2,r}\left[\frac{d(Z_p/M_2)}{dC_2}\right]_r \qquad (5.4)$$

Various Derivations for the Extrapolated Molecular Weight

Direct Derivation. Let us consider a homogeneous polymer PX_Z in the presence of a compound BX both of which dissociate as follows:

$$PX_Z \longrightarrow P^{+Z} + ZX^- \qquad (2.0)$$

$$BX \longrightarrow B^+ + X^- \qquad (2.1)$$

For the polymer PX_Z any anion in the solution can act as a common anion. Hence, if water is the solvent or if it is present as a minor component in a solvent system, then the chemical potential of the water molecules and the dissociation products (hydroxyl and hydronium ions) must be considered. The component BX in equation 2.1 can then be replaced by HOH to give the dissociating system: $HOH \rightleftharpoons H^+ + OH^-$. The chemical potential of the water molecules is essentially constant if water is the solvent and, hence, can be neglected. Since equilibrium exists between the hydronium and hydroxyl ions, the activity of the hydronium ions can be expressed as a function of the activity of the hydroxyl ions or vice-versa. Nevertheless, the common ion X^- (or in this case OH^- or H^+) must still be considered with respect to the dissociated polymer. Thus in a dissociating solvent, zero ionic strength is never attained and a system as described in equation 1 becomes incomplete.

The basic equations resulting from equations 2.0 and 2.1 can be shown (13) to be for the polymer PX_Z:

$$d \ln m_P \gamma_{N,P} + Z_P \, d \ln m_X + \ln m_X dZ_P + \left(\frac{1}{RT}\right) dW_{PX_Z} = 2A_{PX_Z} r dr \qquad (3.0)$$

and for the salt BX:

$$d \ln m_B + d \ln m_X + \left(\frac{1}{RT}\right) dW_{BX} = 2A_{BX} r dr \qquad (3.1)$$

at the radius r. Here A_{PX_Z} equals $M_{PX_Z}(1-\bar{V}_{PX_Z}\rho)\omega^2/2RT$. and A_{BX} equals $M_{BX}(1-\bar{V}_{BX}\rho)\omega^2/2RT$. The terms m_P, m_X and m_B express the molalities of P^{+Z}, X^- and B^+, respectively, and $\gamma_{N,P}$ is the nonelectrolyte activity coefficient of the polymer P^{+Z}. Solving equations 3.0 and 3.1 simultaneously gives:

$$\frac{1}{M_{P,r}^{app}} = \frac{1}{M_{D,r}} + B_{D,r} C_{P,r} \qquad (4.0)$$

where

$$M_{D,r} = M_{PX_Z}\left[1 - Z_{P,r}\left(\frac{M_{BX}}{M_{PX_Z}}\right)\left(\frac{1-\bar{V}_{BX}\rho}{1-\bar{V}_{PX_Z}\rho}\right)\right] \qquad (4.1)$$

and

$$B_{D,r} = -\left(\frac{Z_P}{M_D}\right)_r \left(\frac{d \ln C_B}{d C_P}\right)_r + \left(\frac{1}{RT}\right)\left(\frac{dW_D}{M_D dC_P}\right)_r + \left(\frac{d \ln \gamma_{N,P}}{M_D dC_P}\right)_r + k_{Z,r}\left[\frac{d(Z_P/M_D)}{dC_P}\right]_r \qquad (4.2)$$

where

$$k_{Z,r} = \ln\left[(C_B/M_B) + (Z_P C_P/M_P)\right]_r$$

II. EXTRAPOLATED MOLECULAR WEIGHT

Introduction

Previously (10, 13), equations were derived for the extrapolated molecular weight of an electrostatically charged polymer for equilibrium ultracentrifugation studies. In the presence of a salt BX the equation for the $[PX_Z - (Z_p/2)BX]$ component (equations 6, 25 and 27 of reference 10) relates the extrapolated molecular weight M^{ext} to the factor $(Z_p/2)M_{BX}Q$ while the equation for the polymer itself (see equation 7.0 of Part I) relates M^{ext} to $(Z_p)M_{BX}Q$. Here Q is $1-\bar{v}_s\rho/1-\bar{v}_p\rho$ and Z_p is the charge of the polymer. In addition, Lamm (33) and Johnson et al. (26, 25, 24) also obtained the quantity $(Z_p/2)M_BQ$ in their derivations. It will be shown in this paper that the correct value should be a function of Z_p and not $(Z_p/2)$, i.e., $M^{ext} = M_p[1 - (Z_p)(M_{BX}/M_{PX_Z})Q]$.

In the absence of added salt Pedersen (47) and Johnson et al. (26, 25, 24) have stated that the extrapolated molecular weight of a charge polymer should be $M_{PX_Z}/(1 + Z_p)$ where M_{PX_Z} is the true molecular weight of the polymer PX_z having a charge Z_p and counterion X^-. However, Pedersen qualified this result by stating that this is true when the polymer PX_z is the only electrolyte present in the solution. The conclusion (26, 25, 24) that in the absence of salt an extremely low extrapolated molecular weight will result is based on the assumption that the molality of the charged polymer m_p and that of its counterion m_x can be treated as a thermodynamically independent species in a polar solvent:

$$d \ln a_{px_z} = d \ln m_p m_x^z \bar{\gamma}_p \bar{\gamma}_x^z = (Z+1) d \ln m_{\pm px_z} \bar{\gamma}_{\pm px_z} = 2A_{px_z} r dr \qquad (1)$$

Here A_{px_z} equals $M_{px_z}(1 - V_{px_z}\rho)\omega^2/2RT$. (24) Also a_{px_z} is the activity of the charged polymer PX_z, m_p and m_x are the molalities of the polymer and its counterion and $\bar{\gamma}_p$ and $\bar{\gamma}_x$ are their respective activity coefficients. Lamm (33) has pointed out in the discussion of his equation 19 that the term $(Z_p/2)M_{BX}Q$ is independent of the concentration of the salt and the concentration of the polymer. That is, this term exists at both finite and infinitely dilute concentrations of salt or polymer. Hence, any ion which can be exchanged between the polymer and solute—and thus act as a common ion—must be considered even though the concentration of the ion or that of the polymer is infinitely dilute. Because dissociated solvent molecules, as well as stray ions such as those formed from dissolved gases, can fulfill this requirement, the approach given in equation 1 is not applicable to charged polymer solutions.

When such ions are considered, the molalities m_p and m_x must be handled separately. Then the theoretical molecular weight obtained at zero polymer concentration in the absence of a supporting electrolyte is the same as that obtained in the presence of a salt; that is, the supporting electrolyte can be assumed to be present in infinitesimal amounts. In this part the approach by Johnson et al. (24) as modified by Erlander (10), the approach by Williams et al. (55), the approximation given by Lamm (33) and a more direct formulation (13) will be reviewed in order to show how these various results were obtained and, hence, clarify the discrepancies among them.

($C_{B,a}/C_{B,b}$) if there are only a few ions present such as those due to dissolved carbon dioxide or trace salts. As the concentration of salt is increased, the effect of the polymer on ($C_{B,a}/C_{B,b}$) will decrease, i.e., will be "swamped" out, and, hence, ($C_{B,a}/C_{B,b}$) should approach one [or log ($C_{B,a}+C_{B,b}$) should approach zero]. In other words, as the ratio (C_{BX}/C_{PX_z}) is increased, the concentration gradient of the low molecular weight electrolyte should approach that obtained in the absence of polymer. As the molecular weight of BX increases, the ability of the polymer to change the concentration of BX may decrease. Also the interaction of the ions B^+ and X^- with the polymer should depend on the ratio of charge to mass for both the polymer and the ions B^+ and X^-. Consequently, a decrease in the molecular weight of the salt BX and an increase in the ratio of charge to mass for its ions B^+ and X^- may yield a greater deviation in the concentration of BX in the polymer solution then in the corresponding solvent solution, i.e., may increase the value of the B coefficient. Furthermore, the addition of the salt will reduce electrostatic repulsion between polymer units and, hence, will allow the polymer to have a greater concentration at the cell bottom, i.e., the value ($C_{p,b} - C_{p,a}$) will increase giving a decrease in B.

The purpose of substituting the refractive increment (dn/dr) for (dC/dr) in the ultracentrifugal equations of Williams et al. (55), was to apply the experimental data directly to the theoretical equations. The polymer has been redefined by Johnson et al. (24), in order again to determine the concentration of the polymer from the schlieren or interference optical systems with more precision. With the introduction of the absorption system according to the method of Schachman (43), the concentration of the polyelectrolyte may be obtained directly. Hence, the previous errors introduced by refractive increment methods can be eliminated. Furthermore, the magnitude of such errors can be estimated by comparing the two methods.

Synopsis

The basic equations for equilibrium ultracentrifugation have been examined. The method given by Goldberg was used to derive general equations for the molecular weight of a polyelectrolyte having q components. The results were applied to a homogeneous polyelectrolyte PX_z, which exists in solution in the presence of a lower molecular weight electrolyte BX. In this application it was assumed that the activity coefficients of the ions B^+ and X^- are constant, that the cross-coefficients are negligible and that the binding between salt and polymer is absent. The resulting equations show that the electrical potential and the possible change in the electrostatic charge of a polymer with a change of polymer concentration must be considered. The electrical potential term as manifest in the polarization or dipole moment of the polymer may account for the low "effective" electrostatic charge observed not only in ultracentrifugal measurements, but also in other physical studies of polyelectrolytes.

be shown* that the value of C_2 can be defined as $C_2 = C_P [1 - (M_B/2)(Z_p/M_p)Q]$ where $Q = (1-\bar{V}_B\rho')/(1-\bar{V}_P\rho)$. Only when the charge Z_p is constant will dC_2 be proportional to dC_p. When the charge Z_p varies, then $dC_2 = [(1 - qZ_p)dC_p - qC_p dZ_p]$ where $q = (M_B Q/2M_p)$. Thus substitution of C_p for C_2 into equation 5 of reference 10 would involve all the terms in the B coefficient. Consequently the coefficient of the term $d(Z_p/M_p)/dC_p$ as given in equation 8 is always negative, whereas the coefficient of this same term in equation 5 of reference 10 is always positive. For example, the value of the coefficient

$$(1/2)\left\{\ln [1 + f] + (1/[1 + (1/f)])\right\}$$

for the (dZ_p/dC_2) term where $f = (Z_p/M_2)(M_B C_2/C_B)$ as given in equation 5 of reference 10 ranges from zero at $C_2 = 0$ to 1.26 for the conditions where $C_2 = 0.23\%$, $(Z/M)_p = (20/69)\ 10^{-3}$ and $(C_B/M_B) = m_B = 1.5 \times 10^{-7}$ for a BSA solution in HCl at pH 3.8 (12). The corresponding change in the $d(Z/M)_p/dC_p$ coefficient of equation 8 is (-16) at $C_p = 0$ to (-14) at $C_p = 0.23\%$. It is therefore concluded that the B_D coefficient of equation 8 expresses more directly and accurately the concentration dependence of a charged polymer when the charge Z_p varies with the cell radius. This variation apparently occurs when, for practical purposes, only an acid is present (12).

Let us consider the term $(-d \ln C_B/d C_p)$ in the B_D coefficient as given in equation 7.1. Since this term must be positive for a positive B_D coefficient, then the concentration of the low molecular weight electrolyte BX must be lower at the cell bottom than at the meniscus. This effect is opposite to the changes in concentration occurring with a freely sedimenting species. Since the solvent pattern is subtracted from the solution pattern in sedimentation-equilibrium calculations, then the change in C_B as observed in the B_D coefficient may be the difference between the actual changes in the salt BX occurring in the solution and solvent. To the author's knowledge this possibility has never been examined. Nevertheless the increase in the concentration of salt at the meniscus can be explained on the basis of a Donnan equilibrium effect (23) involving the increase in the concentration of the polymer with cell radius.

The effect of salt on the B_D coefficient can be examined by considering the $(-d \ln C_B/dC_P)$ term. This will be the main positive term in the B_D coefficient for most polyelectrolytes since the $d(Z_p/M_p)$ term appears to be zero in the presence of a small amount of salt (0.0005 molar) (12) and since the nonelectrolyte coefficient γ_P depends on the molecular configuration and is most likely small. Integration of the $(-d \ln C_B/dC_P)$ term yields the expression: $2.303\ (Z_p/M_D)[\log (C_{B,a}/C_{B,b})]/(C_{p,b} - C_{p,a})$. The macroelectrolyte can effect a greater change in the ratio

* The molality of the $[PX_z - (Z/2)BX]$ component, i.e., the component -2, should equal the molality of the polymer $(m_2 = m_p)$. If we assume that molalities are equal to molarities, then $(C_2/M_2) = C_p/M_p)$ or $C_2 = C_p(M_2/M_p)$. Thus, the relationship of the concentration C_2 for the polymer $[PX_Z - (Z/2)BX]$ to the polymer concentration C_p is: $C_2 = C_p [1 - (M_B/2)(Z'/M'_p)Q]$ using the definition of M_2 as given in reference 10.

appear that each charged polyelectrolyte must contribute in some manner to the ionic strength. In addition, at low salt concentrations, the interaction between like charges attached to the same polyelectrolyte may not be compensated sufficiently by the swelling of the polymer. Hence, as the concentration of the low molecular weight electrolyte is reduced, the net charge Z_p on the high molecular weight electrolyte may approach the behavior of a single ion having a charge Z_p. In contrast, at high salt concentrations each charge on the polymer—although restricted in its movement—may behave as a point charge, i.e., as a separate entity apart from the polymer molecule.

The main term in the expression for the electrical potential term dW_D of equation 8 is the dipole moment coefficient γ_d and not the electrical potential coefficient γ_E. Since the dW_D term in the B coefficient will be negative, then any polyelectrolyte which has a dipole moment will have a lower concentration dependence than that predicted by equation 1. Hence, the use of the term "effective" charge (see Introduction) may not be completely accurate. That is, the dipole moment of a polyelectrolyte may be the main factor in reducing the concentration coefficient B. Incorporation of the dW_D term may therefore make the effective charge equal to the charge obtained from titration studies. With regard to this point, Scatchard and Bregman (45) concluded that polarization of the protein molecule decreased the B coefficient as obtained from light-scattering studies. Hence, polarization (induced or permanent dipole moments) may be involved in physical studies other than ultracentrifugation.

In equation 4.0 and subsequent equations it was assumed that at a specific radius all molecules of the homogeneous polymer have the same charge $Z_{P,r}$. However, the existence of the $d(Z_{p,r}/M_p)/dC_{P,r}$ term in equations 8 and 7.1 suggests that the charge $Z_{P,r}$ could vary with the radius. For example, the ratio of the buffer to the polymer will be greater at the meniscus than at the cell bottom because the change in concentration with r will be greater for the higher molecular weight species (the polymer) than for the low molecular weight electrolyte. In addition, the concentration of the salt may be greater at the meniscus than at the cell bottom due to osmotic pressure effects (see Part II). If only a strong acid is essentially present, e.g., hydrochloric acid, the change in the concentration of the acid with cell radius will also induce a corresponding change in the pH. When the pH is in the neighborhood of the pK of carboxylate or other groups, then this change in pH will change the electrostatic charge Z_r with the cell radius. A change in pH with cell radius may not arise if sufficient salt is present since then the cation of the salt could replace the hydronium ion, which in turn could migrate freely. Thus, in the absence of added salt, the charge $Z_{P,r}$ may vary with the cell radius.

In a previous paper (10) equations were derived that involved a change in the value of (Z/M) with a change in the concentration of the component $[PX_Z - (Z/2)BX]$, i.e., a change in the value of C_2. It will now be shown that one must consider the expression for d(Z/M) as given in equations 7.1 and 8 rather than that for the $[PX_Z - (Z/2)BX]$ component as given in equation 5 of reference 10. The definition of C_2 involves the electrostatic charge $Z_{P,r}$ which has now become a variable. That is, it can

The subscripts specify the species being considered. Assuming that $m_x = m_B + Z_p m_p$ and that molalities are equal to molarities, i.e., $m_p = C_p/M_p = C_{PX_Z}/M_{PX_Z}$ and $m_B = C_B/M_B = C_{BX}/M_{BX}$, then equation 5 becomes:

$$2A_{D,r} = 2\left[A_{PX_Z} - Z_{p,r}A_{BX}\right] = \left(\frac{1}{C_{p,r}}\right)\left(\frac{dC_{p,r}}{r\,dr}\right)\Big/\left(1 + B_{D,r}M_{D,r}C_{p,r}\right) \tag{6}$$

at the radius r. Here the value of $M_{D,r}$ is

$$M_{D,r} = \frac{2RTA_{D,r}}{(1-\bar{V}_p\rho)\omega^2} = M_{PX_Z}\left[1 - Z_p\left(\frac{M_{BX}}{M_{PX_Z}}\right)\left(\frac{1-\bar{V}_{BX}\rho}{1-\bar{V}_{PX_Z}\rho}\right)\right] \tag{7.0}$$

where it is assumed that $V_D = V_p$. The value of $B_{D,r}$ is

$$B_{D,r} = -\left(\frac{Z_p}{M_D}\right)_r\left(\frac{d\ln C_B}{dC_p}\right)_r + \left(\frac{1}{RT}\right)\left[\frac{dW_D}{M_D\,dC_p}\right]_r + \left(\frac{d\ln\gamma_{N,p}}{M_D\,dC_p}\right)_r + \Big\}\ln\left[\left(\frac{C_B}{M_B}\right) + \left(\frac{Z_pC_p}{M_p}\right)\right]\Big|_r\left[\frac{d(Z_p/M_D)}{dC_p}\right]_r \tag{7.1}$$

In terms of molecular weights equation 6 becomes,

$$\frac{1}{M_{p,r}^{app}} = \frac{1}{M_{D,r}} + B_{D,r}\,C_{p,r} \tag{8}$$

where

$$M_{p,r}^{app} = \left(\frac{1}{C_{p,r}}\right)\left(\frac{dC_{p,r}}{r\,dr}\right)\left[\frac{RT}{(1-\bar{V}_{PX_Z}\rho)\omega^2}\right]$$

The subscript "D" has been added to designate this direct derivative. In addition, the subscript r emphasizes that these equations are valid only at a specific radius r. The values of $M_{D,r}$ and $B_{D,r}$ are defined as given in equations 7.0 and 7.1. The quantities dW_D and W_i are given in equations 5, 3.5, and 2.4. The concentration C_p of the polymer is expressed in units of weight per unit volume.

Discussion

The B coefficient as expressed in equations 7.1 and 8.0 for the molecular weight determinations includes two additional terms that were not considered previously (55): the term expressing the electrostatic potential dW_D and the term involving a variation in the electrostatic charge $d(Z_p/M_D)$. The electrostatic potential will change with a change in the ionic strength of the medium. Both the high and low molecular weight electrolytes should contribute to the ionic strength. Hence the electrical potential term of a polyelectrolyte is always present because sedimentation-equilibrium runs are based on the fact that the concentration of the electrolyte changes from the meniscus to the cell bottom.

If the molecular weight of the high molecular weight electrolyte is large, then the process by which the polyelectrolyte contributes to the ionic strength becomes complex. That is, the size of the molecule—to which the electrostatic charge is attached—produces steric hindrances when this charge and like charges try to form a distribution around another given charge. Although such complications arise in calculating the interaction between polyelectrolyte molecules, nevertheless it would

the same. For complete dissociation of PX_z and BX the activities a_{PX_z} and a_{BX} become $a_{P^{+z}}a_{X^-}^{z}$ and $a_{B^+}a_{X^-}$, respectively. For brevity the subscripts P^{+z}, X^- and B^+ will be designated P, X and B. Let us assume that binding between all ions is completely absent. Then, since we are considering here only the salt plus a single polymer species PX_z, the activity coefficient terms in equation 3.5 or 3.6 become:

$$\sum_{j=1}^{q+1}\left(\frac{\partial \ln \gamma_{N,i}}{\partial m_j}\right)_{T,p,m_k} dm_j = \left(\frac{\partial \ln \gamma_{N,i}}{\partial m_{PX_z}}\right)_{T,p,BX} dm_{PX_z} + \left(\frac{\partial \ln \gamma_{N,i}}{\partial m_{BX}}\right)_{T,p,PX_z} dm_{BX}$$

where i = PX_z or BX. Equations 3.5 and 3.6 can thus be simplified by assuming that the terms $\gamma_{N,B}$ and $\gamma_{N,X}$ are constant and that the interaction of the salt and polymer is negligible, i.e., the cross coefficient terms $(\partial \ln \gamma_{N,PX_z}/\partial m_{BX})_{T,p,PX_z}$ and $(\partial \ln \gamma_{N,BX}/\partial m_{PX_z})_{T,p,BX}$ are zero for the nonelectrostatic coefficient. Thus, the term $\Sigma(\partial \ln \gamma_{N,PX_z}/\partial m_j)_{T,p,m_k} dm_j$ becomes equal to d ln N, PX_z. Since m_{PX_z} equals $m_P m_X^z$; then equation 3.5 becomes for the polymer PX_z:

$$d \ln m_P \gamma_{N,p} + d\left(\ln m_X^{Z_p}\right) + \left(\frac{1}{RT}\right) dW_{PX_z} = 2 A_{PX_z} r dr$$

or

$$d \ln m_P \gamma_{N,p} + Z_p d \ln m_X + \left(\ln m_X\right) dZ_p + \left(\frac{1}{RT}\right) dW_{PX_z} = 2 A_{PX_z} r dr \qquad (4.0)$$

and for the buffer BX equation 3.6 becomes:

$$d \ln m_B + d \ln m_X + \left(\frac{1}{RT}\right) dW_{BX} = 2 A_{BX} r dr \qquad (4.1)$$

where A_{Bx} and A_{px_z} are defined as A_i in equation 3.5. Except for the electrostatic term, equations 4.0 and 4.1 are similar to equations used previously (55, 37, 10). Equations 2.0, 2.1, 4.0 and 4.1 have been examined by Williams et al. (55). In their derivation they did not consider the electrostatic term dW or any varition in the charge Z_p. (See equation 55 of reference 55.)

The charge Z_p determines the concentration of anion (or cation) released from the polymer. As pointed out by others (55, 33, 24) and in equations 2.0 and 2.1, the molality of the anion (X^-) is $m_x = m_{BX} + Z_p m_{PX_z}$. Hence a variation in the charge $Z_{P,r}$ will not only change the coefficient of the d ln m_x and dW_{PX_z} terms but will also effect a change in the concentration of (X^-). Consequently, the possibility that the electrostatic charge is a variable must be considered in equation 4.0.

If the term d ln m_x is eliminated between equations 4.0 and 4.0 as suggested by Williams et al. (55), in deriving their similar equations, then,

$$d \ln m_p \gamma_{N,p} + \ln m_X \, dZ_p - Z_p \, d \ln m_B + \left(\frac{dW_D}{RT}\right) = 2\left(A_{PX_z} - Z_p A_{BX}\right) r dr \qquad (5)$$

where

$$dW_D = d\left(W_{PX_z}\right) - Z_p \, d\left(W_{BX}\right).$$

Substitution of dp from equation 3.1 and $d\mu_i$ from equation 3.0 into equation 3.2 gives:

$$M_i\omega^2 r dr - M_i d(W/M)_i = M_i \bar{V}_i \Big\} \rho\omega^2 r dr - \rho' d(W/M)_{P_i X_{Z_i}} - \rho'' d(W/M)_{BX} \Big(+ \sum_{j=1}^{q+1} \left(\frac{\partial \mu_i}{\partial m_j}\right)_{T,p,m_k} dm_j \qquad (3.3)$$

since $(\partial\mu_i/\partial p)_{T,m}$ is the partial molal volume $M_i V_i$. (22) Rearrangement of equation 3.3 gives:

$$M_i(1-\bar{V}_i\rho)\omega^2 r dr = \sum_{j=1}^{q+1} \left(\frac{\partial \mu_i}{\partial m_j}\right)_{T,p,m_k} dm_j + (\nu) dW_i \qquad (3.4)$$

where

$$(\nu) = \Big\} 1 - \bar{V}_i\rho' \left[\frac{d(W/M)_{PX_Z}}{d(W/M)_i}\right] - \bar{V}_i\rho'' \left[\frac{d(W/M)_{BX}}{d(W/M)_i}\right] \Big($$

Since ρ' and ρ'' are the densities of the polymer and the buffer per <u>total</u> volume, they will be much less than one. Hence, it can be assumed that (v) = 1 in equation 3.4.

The chemical potential can be written in terms of the molal concentration m_i and the activity coefficient $\gamma_{N,i}$ as given in equation 2.2. If there is no binding or complexing between all q components of the heterogeneous polymer, then equation 3.5 becomes for the $i^{\underline{th}}$ polymer species:

$$2A_i r dr = d\ln m_i + \sum_{\substack{j=1 \\ j=BX}}^{q} \left(\frac{\partial \ln \gamma_{N,i}}{\partial m_j}\right)_{T,p,m_k} dm_j + \left(\frac{1}{RT}\right) \left(\frac{\partial \mu_i}{\partial m_{BX}}\right)_{T,p,m_k} dm_{BX} + \left(\frac{1}{RT}\right) dW_i \qquad (3.5)$$

where $A_i = \dfrac{M_i(1-\bar{V}_i\rho)\omega^2}{2RT}$ and where $i = P_i X_{Z_i}$. If there is no binding or complexing between the ions of BX, then equation 3.4 becomes for the low molecular weight electrolyte:

$$2A_{BX} r dr = d\ln m_{BX} + \left(\frac{\partial \ln \gamma_{N,BX}}{\partial m_{BX}}\right)_{T,p,m_k} dm_{BX} + \left(\frac{1}{RT}\right) \sum_{\substack{j=1 \\ j=BX}}^{q} \left(\frac{\partial \mu_{BX}}{\partial m_j}\right)_{T,p,m_k} dm_j + \left(\frac{1}{RT}\right) dW_{BX} \qquad (3.6)$$

The quantity $(\partial \mu_{P_i X_{Z_i}}/\partial m_{BX})_{T,p,m_k}$ is a function of the binding coefficient Γ where $\Gamma = (\partial m_{P_i X_{Z_i}}/\partial m_{BX})_{T,p,\mu_k}$. (7) The electrostatic term $d W_{P_i X_{Z_i}}$ or $d W_{BX}$ is a function of the change in the ionic strength with radius. Both the low molecular weight electrolyte and the high molecular weight electrolyte contribute to the ionic strength. The electrostatic and binding terms will be discussed in more detail in Parts IV and V.

Concentration Coefficient B for Homogeneous Polyelectrolyte

Consider a homogeneous polymer, PX_Z, such as a protein, and a low molecular weight polyelectrolyte, BX, which both dissociate according to equations 2.0 and 2.1. If the sign of the electrostatic charges are the opposite of those designated in equations 2.0 and 2.1, the results remain

$\bar{\gamma}_i = \gamma_{N,i}\gamma_{E,i}\gamma_{d,i}$. Rewriting equation 2.2 in terms of these coefficients gives:

$$\bar{\mu}_i = \mu_i + W_i \qquad (2.3)$$

For the polymer $\mu_i = \mu_{P_iX_{Z_i}} = \mu^{\circ}_{P_iX_{Z_i}} + RT \ln m_{P_i^{+z_i}} m_{X^-}^{Z_i} \gamma_{N,P_i^{+z_i}}^{} \gamma_{N,x^-}^{Z_i}$

and $W_i = W_{P_iX_{Z_i}} = RT \ln \gamma_{E,P_i^{+z_i}} \gamma_{E,X^-}^{Z_i} \gamma_{d,P_i^{+z_i}} \gamma_{d,X^-}^{Z_i}$

For the salt $\mu_i = \mu_{BX} = \mu^{\circ}_{BX} + RT \ln m_{B^+} m_{X^-} \gamma_{B^+} \gamma_{X^-}$ and $W_i = W_{BX} = RT \ln \gamma_{E,B^+} \gamma_{E,X^-} \gamma_{d,B^+} \gamma_{d,X^-}$

The term $RT \ln \gamma_{E,i}$ is the electrical energy resulting from the addition to the solution of one mole of the $i^{\underline{th}}$ type ion (5). This reasoning also applies to $RT \ln \gamma_{d,i}$. The electrostatic charge Z_i is the total number of positive (or negative) charges minus the total number of negative (or positive) charges, i.e., the net charge. The equal number of + and - charges can be considered as zwitterions that give rise to the dipolar effect $RT \ln \gamma_{d,P_i^{+z_i}} \gamma_{d,X^-}^{Z_i}$. According to Williams et al. (55), it is the total potential U_i for each particular molecular species that is constant in all phases at equilibrium. This total potential at the radius r using equation 2.3 is:

$$U_i = \mu_i - \left(\frac{M_i \omega^2 r^2}{2}\right) + W_i \qquad (2.4)$$

The method of Goldberg (22) can be applied directly to the system given in equation 2.4. Since the total potential U_i for heterogeneous equilibrium is constant, then at the radius r:

$$dU_i = d\mu_i - M_i \omega^2 r\, dr + dW_i = 0 \qquad (3.0)$$

Here dW_i can also be expressed as $M_i d(W_i/M_i)$. The chemical potential μ_i at constant temperature and at the radius r is related to the pressure by the Gibbs-Duhem equation $V dp = \sum_{i=0}^{q+1} X_i d\mu_i$ where X_i is the number of moles of component i in the phase R of volume V and dp is the change in pressure (22). Substitution of $d\mu_i$ from equation 3.0 into this expression gives:

$$dp = \left(\frac{\sum_{i=0}^{q+1} M_i X_i}{V}\right) \omega^2 r\, dr - \left(\frac{\sum_{i=0}^{q+1} M_i X_i d(W/M)_i}{V}\right) = \rho \omega^2 r\, dr - \rho' d(W/M)_{P_iX_{Z_i}} - \rho'' d(W/M)_{BX} \qquad (3.1)$$

assuming that $(W/M)_{P_iX_{Zi}}$ is the same for all P_iX_{Zi} species. The summation from i = 0 to i = (q + 1) includes the solvent, the salt BX and all polymer species. Thus $\rho = \sum_{i=0}^{q+1} M_i X_i / V$ is the density of phase r, ρ' is the density of the polymer PX_Z, and ρ'' is the density of the buffer BX per total volume assuming that d(W/M) is zero for the solvent molecules. The chemical potential at the radius r and at constant temperature can be expressed as a function of the pressure and the molal concentration m_j of all species:

$$d\mu_i = \left(\frac{\partial \mu_i}{\partial p}\right)_{T,m} dp + \sum_{j=1}^{q+1} \left(\frac{\partial \mu_i}{\partial m_j}\right)_{T,p,m_k} dm_j \qquad (3.2)$$

For example, the electrostatically charged polymer Proteus vulgaris flagellin has essentially no concentration dependence even under conditions where the ratio of charge to mass is relatively large (11). In addition, bovine serum albumin (BSA) has much lower concentration dependence than would be predicted from equation 1 if the charge as obtained by titration studies is employed. Also, the discrepancies between observed and theoretical (eq. 1) values varies with the polymer, being greater for flagellin than BSA.

An "effective" charge, which is much lower than the real charge, has been employed by Stigter (46) and Prins et al. (40), in light-scattering studies. Similarly, Mijnlieff (37, 38) postulated that the electrical double layer considerably reduces the real charge in sedimentation and diffusion studies. Electrophoresis and osmotic pressure studies (39) also suggest that an effective charge is much lower than the real charge of a polyelectrolyte. However, the discrepancies between theoretical and observed values for osmotic pressure results as summarized by Rice and Nagasawa (41) and for ultracentrifugation results suggests that the structure of the polymer determines the magnitude of the difference between the effective and real charge. In order to enlighten further this charge effect, the basic equations for equilibrium ultracentrifugal molecular weight determinations are examined.

General Equations

Let us consider the ultracentrifuge equilibrium distribution of a system containing a macroelectrolyte, $P_iX_{z_i}$, containing q species plus a low molecular weight electrolyte, BX, in a solvent where the salt and the polymer dissociate as follows (50, 24, 10):

$$P_iX_{z_i} \longrightarrow P_i^{+z_i} + z_iX^- \qquad (2.0)$$

$$BX \longrightarrow B^+ + X^- \qquad (2.1)$$

Here Z_i is the net charge on the polymer. Other charges that exist can be considered as zwitterions. The chemical potential μ_{BX} of a salt is equal to the sum of the chemical potentials of its ions. For brevity, the chemical potential of the salt is treated, i.e., μ_{BX} and $\mu_{P_iX_{z_i}}$ instead of $\mu_{B^+} + \mu_{X^-}$ and $Z\mu_{X^-} + \mu_{P_i}^{+z_i}$. The chemical potential of the polymer and that of the low molecular weight electrolyte can then be written (5):

$$\bar{\mu}_i = \mu_i^\circ + RT \ln \bar{a}_i \qquad (2.2)$$

where μ_i° is a constant and where $\bar{a}_i$ is the activity of the $i^{\underline{th}}$ species, i.e., i = BX or $P_iX_{Z_i}$. For the dissociated polymer or salt, the activity can be expressed as $\bar{a}_i = m_i m_{x^-}^{z_i} \bar{\gamma}_i \bar{\gamma}_{x^-}^{z_i}$ where m is the molality and $\bar{\gamma}$ is the activity coefficient and where $Z_i = 1$ for the salt. The activity coefficient $\bar{\gamma}_i$ involves the concentration dependence of the $i^{\underline{th}}$ species as a nonelectrolyte ($\gamma_{N,i}$) and in addition, that due to the interaction of electrostatic charges ($\gamma_{E,i}$) and the dipole moment ($\gamma_{d,i}$). Therefore, we can assume that the activity coefficient is the product of the three coefficients:

Part V discusses the possible effect that the ionic atmosphere of a polyelectrolyte may have on the concentration coefficient and the extrapolated molecular weight. The binding coefficients as developed in the general equations of Part I are applied to a homogeneous polymer PX_z.

This review is restricted to specific problems of the ultracentrifuge. More general reviews have occurred in the literature which will give the reader a broader scope of the treatment and application of ultracentrifuge data. The most notable review concerning equilibrium and sedimentation theory is that of Williams, Van Holde, Baldwin and Fujita (55). An extension has been given by Baldwin and Van Holde (2). Another review was written by Williams (56) with regard to charge effects of polyelectrolytes during sedimentation ultracentrifugation. Fujita (21) and Schachman (42) have each written a book concerning a general review of ultracentrifugation. Also, the correlation between osmotic pressure, light scattering and ultracentrifugation has been treated briefly by Eisenberg (6, 7) and Casassa and Eisenberg (3), in addition to that given by Goldberg (22) and Fujita (20). These reviews, however, do not treat the equilibrium ultracentrifugation of polyelectrolytes in detail and omit some of the factors included in this review.

I. BASIC EQUATIONS

Introduction

The determination of the molecular weight of a charged polymer by the sedimentation-equilibrium method has been examined briefly by Svedberg and Pedersen (47), Tiselius (48), Lamm (33) and more recently in detail by Johnson et al. (24), and Williams et al. (55). An extension of the method of Johnson et al. (24) was made in an earlier paper (10). At present, the exact effect of salt in reducing the concentration dependence or in determining the extrapolated sedimentation-equilibrium molecular weight of a charged polymer is not clearly understood. Lamm (33) had pointed out that if the charge to weight ratio of the polymer is large, the addition of salt as a supporting electrolyte will not produce a solute that can be treated as if it were neutral. Hence, the concentration coefficient of an electrostatically charged polymer must be examined even when the polyelectrolyte is present with large amounts of salt.

In developing an equation for the molecular weight as a function of polymer concentration C_p, various assumptions have been made (50, 33, 24). All resulting equations (50, 33, 24) are essentially the same and can be expressed at the radius r as:

$$\frac{1}{M_r^{app}} = \frac{1}{M^{ext}} + B_r C_{p,r} \tag{1}$$

where

$$B_r = \left(\frac{1}{2}\right)\left(\frac{Z_p^2}{M_p M^{ext}}\right)\left(\frac{M_B}{C_B}\right)$$

and where M_p, M_r^{app} and M_r^{ext} are the true, the apparent and the extrapolated molecular weights. However, some experimental data give concentration coefficients which are much lower than that predicted by eq. 1.

Because many completely dissociated proteins cannot exist in aqueous solutions in the presence of large amounts of salt, the value of the extrapolated molecular weight in the absence of added salt is both a theoretical and a practical subject. At present, a controversy (26) exists as to whether the extrapolated molecular weight M^{ext} in the absence of added salt is $(1 + Z_p)^{-1}$ times the true molecular weight M_p or whether it is more nearly equal to M_p. Light scattering data (32) suggests that the relationship $M^{ext} = M_p/(1 + Z_p)$ is not correct while ultracentrifuge data (26) indicates that it is valid. In the discussion given in Part II it is shown that the ultracentrifuge data can be interpreted in a manner which contradicts the relationship $M^{ext} = M_p/(1 + Z_p)$. The equations leading to the relationship $M_p/(1 + Z_p)$ are obtained by assuming that a polyelectrolyte in a polar solvent can be treated as a thermodynamically independent species apart from any other existing trace ions. The basis for invalidating the above expression is that a solvent system which is free from all electrolytes other than the polymer can never be attained and that these ions must be considered. Hence, a concept which has been held to be true almost since the advent of the ultracentrifuge is questioned. In addition, a survey of the literature supports the conclusions made concerning the value of the extrapolated molecular weight as obtained with (or without) added salt.

Most proteins or polyelectrolytes are heterogeneous to some extent. Therefore, equations which relate the B coefficient and the extrapolated molecular weight to heterogeneous polymers are important. The B coefficient (equation 7.1 of Part III), as well as equations for $\bar{M}_Z$, $\bar{M}_W$ and $\bar{M}_n$ (equations 15, 18 and 24 of Part III), can be applied to both charged and uncharged polymers.

To the author's knowledge, the electrical potential term in the equations for equilibrium ultracentrifugation has not been treated in the literature. The equations developed in Part IV, therefore, represent a new concept with regard to interpretation of the B coefficient. They suggest that if a polymer has a dipole moment, then the concentration dependence of that polymer can be reduced drastically. A dipole moment may be permanent or induced. Permanent polarization can be related to the spatial arrangement of different charged groups while induced polarization can be related to the movement of ions across the surface of a polyelectrolyte as one polymer ion approaches another. In treatment of the electrical potential term, it is assumed that the polyelectrolyte being studied contributes to the ionic strength. The concentration gradient of the polymer formed in the ultracentrifuge cell therefore induces a change in the electrical potential with cell radius. As the polymer's molecular weight and electrostatic charge become larger and its secondary and tertiary structure more intricate, the effect that a polymer has on the ionic strength becomes more complex. Nevertheless, the charge of a polyelectrolyte can not be ignored and must be considered as contributing to the ionic strength in some manner. If a polyelectrolyte has both positive and negative charges on its surface, as in the case of most proteins, then such an array of zwitterions will most likely produce a dipole moment. A dipole moment may exist even when the net charge of the polymer is zero. As discussed in Part IV, the existence of a dipole moment on a neutral polymer may effect the extrapolated molecular weight but most likely will not contribute to the concentration coefficient.

INTRODUCTION

The ultracentrifuge has become an important tool for analyzing proteins. Originally, Svedburg had intended to use his ultracentrifuge for the determination of size distributions of polymers (56). Later, however, the importance of this instrument for the characterization of proteins became apparent. One method of characterizing is to determine molecular weights by equilibrium ultracentrifugation. Previously, the long time required to attain equilibrium prohibited the application of this method to many proteins. Because of this time factor, the introduction of Archibald's (1) approach-to-equilibrium method by Klainer and Kegeles (31, 28) became popular in a short time. Refinement by the use of the Trautman (49, 9, 69) plot increased this popularity. However, the application of a short column technique (50, 60) revived equilibrium ultracentrifugation as a method for determining the molecular weights of proteins. Thus, at this time, the use of either the equilibrium or the approach-to-equilibrium method for determining the molecular weights of polyelectrolytes has become quite widespread. The equations relating the behavior of charged polymers in equilibrium ultracentrifugation are therefore more important today than ever before.

Despite the wide acceptance of the equilibrium method, confusion appears to exist with regard to the interpretation of the resulting data. Such topics as the extrapolated molecular weight in both the presence and absence of added salt and the exact effect of the electrostatic charge are at present subjected to individual interpretation. Other factors such as the effect of a change in the electrostatic potential with cell radius have been completely ignored. It is the author's intention to bring these and other subjects to the attention of those scientists who apply the existing theoretical equations to their equilibrium or approach-to-equilibrium data on polyelectrolytes.

There appears to be a lack of emphasis and extensive study in the equilibrium ultracentrifugation of polyelectrolytes. In order to emphasize certain topics, this review has been divided into five parts. Each part is an entity by itself, i.e., with its own equations. By presenting the material as separate parts, the author believes that specific points will not be neglected or overlooked. Some mathematical review of the literature is required in order to direct the theory towards specific points and to stress the controversial nature of such subjects as the extrapolated molecular weight.

The first part deals with basic equations and the application of these equations to a homogeneous polyelectrolyte. The general equations were developed by applying the method of Goldberg (22). The treatment differs slightly from that of Goldberg because of the introduction of the electrostatic potential term. In addition, cross-coefficient and ion-binding terms are not neglected. Restrictions are then placed on these general equations in order to emphasize certain basic thoughts. The development of the B coefficient and extrapolated molecular weight for a homogeneous polymer from these general equations is similar to the development of equation 54 of reference 55. The resulting equations for the extrapolated molecular weight and the electrical potential term are discussed as separate topics in subsequent parts.

N as subscript, refers to nonelectrolyte effects

P or P^{+Z} as subscript, refers to high molecular weight electrolyte

Q $(1-\bar{V}_B\rho)/(1-\bar{V}_p\rho)$

q number of molecular species of polymer PX_Z

R gas constant (8.313×10^7 ergs/mole/degree)

r radial distance in cm in ultracentrifuge

$\bar{r}$ distance in cm from center of one ion to center of another ion at its surface; $\bar{r}^{\text{Å}}$ equals this distance in Ångstrom units

S ionic strength of solution

T absolute temperature

U total potential

V volume

$\bar{V}$ partial specific volume

(v) defined in equation 3.4, Part I

W total energy from electrical and dipole moment effects; equals $W_E + W_d$

$W_{E,i}, W_{d,i}, W_{N,i}$ electrical, dipole, or nonelectrical energy resulting from the addition to the solution of one mole of the i^{th} type ion

X or X^- as subscript, refers to the dissociated ion X^- from PX_Z or BX

Z_p net electrical charge of dissociated polymer

Z point charge on ionized low or high molecular weight electrolyte

$\alpha(\rho)$ a function of the radial distances for ionic interactions; equation 14, Part IV

β a constant used in redefining polymer

$\bar{\gamma}$ total activity coefficient

$\gamma_d, \gamma_E, \gamma_N$ activity coefficients pertaining to the dipole moment, electrica charge, and nonelectrostatic effects, respectively

Γ binding coefficient, equation 4, Part V

ρ density of a solution

μ chemical potential per mole excluding electrical and dipole moment effects

$\bar{\mu}$ Total chemical potential per mole including electrical and dipole moment effects

μ dipole moment (equations 14 and 15, Part IV, only)

μ^{D} dipole moment in Debye units

ε charge per mole of protons

θ differential refractive increments on the C scale

λ number of dipolar ions on polyelectrolyte

ψ electrical potential per mole of the phase at r

ω angular velocity

GLOSSARY OF TERMS

A	equals $M(1-\bar{V}\rho)\omega^2/2RT$
a	radial distance to meniscus
a	activity of a salt or ion excluding the electrical and dipole moment effects, γ_E and γ_d
$\bar{a}$	Total activity including all activity coefficients γ_E, γ_d and γ_N; in Part IV only, refers also to distance in cm units between the center of one ion to that of another
$\bar{a}^{\aleph}$	distance in Å units between two ionic centers
B or B^+	as subscript, refers to metallic ion of a soluble low molecular weight electrolyte.
B	concentration coefficient; B_D, B_2, $B^{\aleph}$, $B^{\aleph\aleph}$ and B_Γ are defined by equations 4.2, 5.4, 6.6, 7.2 of Part II and equation 12 of Part V, respectively.
b	radial distance to cell bottom
$\bar{b}$	radius for Kirkwood's spherical model; equation 14, Part IV
C_P or C	concentration in g/ml of polyelectrolyte
C_B	concentration in g/ml of low molecular weight electrolyte
D	dielectric constant (Part IV)
D	as subscript, refers to quantities defined by direct derivation method
d	as subscript, refers to dipolar ion effects
E	as subscript, refers to electrical charge effects
f	defined in equation 5.4 of Part II
G'	a physical factor such as (θ_B/θ_p) or Q which is unity if salt and polymer behave the same
H	a variable defined in equation 9 of Part V
i	as subscript, refers to a particular molecular or ionic species or its property
j	as subscript, refers to a particular molecular or ionic species or to a specific form of the i^{th} molecular species
K	defined as given in equation 6, Part IV
k	1.380×10^{-16}; see equation 15, Part IV
k_E	(0.1644×10^8) $(N\varepsilon^2)$; see equation 8.2, Part IV
k_i	related to activity coefficient, e.g., $k_{N,i} = (\partial \ln \gamma_{N,i}/\partial m_j)_{T,p,m_k}$ (see equation 4.0, Part II)
k_Z	a variable defined in equation 4.2, Part II
L	defined in equation 6.7, Part III
M^{app}	apparent molecular weight as defined in equation 8 of Part I and equations 11.1, 13.1, 15, 19, 22, and 24 of Part III
M^{ext}	extrapolated molecular weight; also designated as M_D, M_2, $M^{\aleph}$, $M^{\aleph\aleph}$, $\overline{M}_{D,W}$, $\overline{M}_{D,Z}$, $\overline{M}_{D,n}$ and M_Γ as defined in equations 4.1, 5.5, 6.5, 7.1, of Part II, equations 25, 26, and 27 of Part III, and equation 15 of Part V, respectively
M_{BX}, M_{PX_Z}	molecular weight of low and high molecular weight electrolytes, respectively
$\overline{M}_n$, $\overline{M}_W$, $\overline{M}_Z$	number, weight and Z-average molecular weights, respectively
m	concentration in molalities
N	Avogadro's number

CONTENTS

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THEORETICAL EQUATIONS FOR ULTRACENTRIFUGAL MOLECULAR WEIGHT DETERMINATIONS OF POLYELECTROLYTES[1]

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ABSTRACT. Both equilibrium and approach-to-equilibrium methods for determining the molecular weights of polyelectrolytes are widely used. However, there exists confusion as to interpretation of data secured. Certain topics as the extrapolated weight with and without added salt and the exact effect of electrostatic charge are subject to individual interpretation. Other factors are often ignored. These and other subjects are brought to the attention of scientists who make use of existing theoretical equations.

The first of the five sections of this review deals with basic equations and their application to a homogeneous electrolyte.

Part II develops the consequences of the finding that completely dissociated proteins cannot exist in aqueous solutions in the presence of large amounts of salt, noting that the extrapolated molecular weight in the absence of added salt is both a theoretical and practical subject.

Part III discusses equations relating to the heterogeneity of polyelectrolytes.

In Part IV are developed equations that include the electrical potential term apparently ignored in the earlier literature. (The dipole moment.)

Part V (The Binding Coefficient) discusses the possible effect that the ionic atmosphere of a polyelectrolyte may have on the concentration coefficient and the extrapolated molecular weight.

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yearling Kamloops trout (Salmo gairdneri) exercised vigorously for 15 minutes at two acclimation temperatures, 11.5°C and 20.0°C. Denyes and Joseph (1956) found no consistent relationship between blood lactic acid concentration of unexercised largemouth bass (Micropterus salmoides) and temperature ranging from 5°C to 35°C.

In July, variability in blood lactic acid level among individual fish was greater than in February-March (Tables 6 and 7, Figures 1 and 2). The highest individual blood lactic acid concentrations were observed in July (Tables 2, 4 and 5). These results may represent an effect of higher temperature but may also be due to a possible insufficient holding period prior to the experiment and a greater difference in voluntary (nonexperimental) and/or experimental activity on the part of individual fish in July.

No significant difference was detected between mean blood lactic acid concentrations of mature male and female carp in the two experiments. Black et al. (1962) noted a consistently higher mean level in both unexercised and exercised female rainbow trout (Salmo gairdneri) than in corresponding groups of males.

An increase in variability among individual fish with increase in the mean blood lactic acid concentration, observed in this study, is evident in the data of Black (1955) as well as many other papers on blood lactic acid in fishes. Because it has not been particularly noted before and may have an important bearing on interpretation of such data, further research on the problem would be useful.

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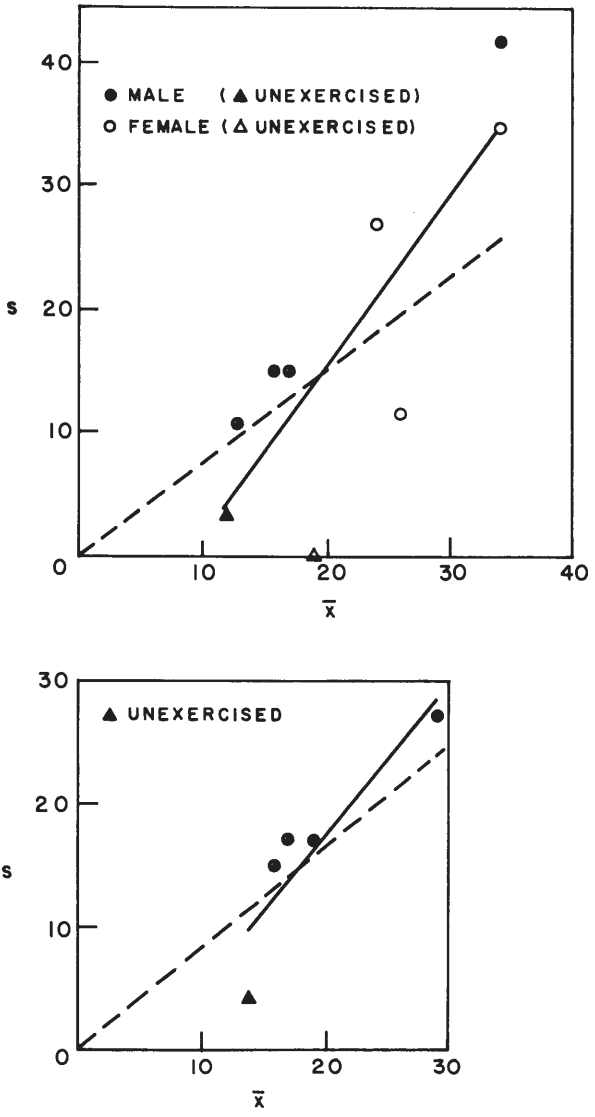

Figure 2. Relationship between standard deviation (s) and mean ($\bar{x}$) blood lactic acid values from mature carp unexercised and during 16 hours after 15 minutes of forced exercise at 24°C—July, 1961 (above, sexes separated; below, sexes combined). [7]

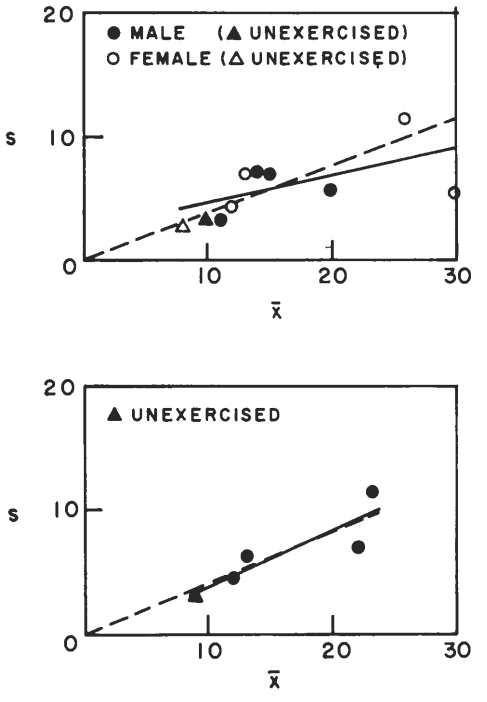

Figure 1. Relationship between standard deviation (s) and mean ($\bar{x}$) blood lactic acid values from mature carp unexercised and during 16 hours after 15 minutes of forced exercise at 1°C—February-March, 1961 (above, sexes separated; below, sexes combined).[7]

[7] The dashed line was fitted as follows: Slope = $\frac{\sum (s/\bar{x})}{N}$, where N is the number of points used in the calculation. The slope of this line represents the average coefficient of variation. The solid line was fitted by the method of least squares. The deviations of the solid line and of the points from the dashed line provide measures of validity of the assumption of relatively constant coefficients of variation.

Table 7. Analysis of variance of transformed blood lactic acid data from mature carp unexercised and during 16 hours after 15 minutes of forced exercise at 24°C—July, 1961.

Source of variation	Degrees of freedom	Sum of squares	Mean square
Sexes	1	0.243315	0.243315
Treatments	4	0.439104	0.109776
Interaction	3*/	0.114151	0.038050
Individuals	33	6.884878	0.208633

$F_{Sexes} = \frac{0.243315}{0.208633} = 1.17$ $F_{0.05(1,33)} = 4.14$

*/ 1 was deducted from the degrees of freedom for interaction for the cell (Female, 16.0-16.4 hours after forced exercise) with no entry.

Table 8. Mean blood lactic acid concentrations of carp unexercised and immediately after 15 minutes of forced exercise at different temperatures (means are followed by standard errors; figures in parentheses represent the range).

Unexercised	Immediately after 15 minutes of forced exercise	Temperature, °C	Study
9 ± 1.0 (6-16)	22 ± 2.3 (11-34)	1	this paper
13.6 ± 0.89 (11.8-17.7)	---	8-10	Secondat and Diaz, 1942
8.5 ± 2.69	54.3 ± 4.49	11.5	Black, 1955
8.5 ± 2.98	77.6 ± 4.33	11.5	Black, 1955
14 ± 1.5 (9-19)	29 ± 9.0 (9-96)	24	this paper

Table 5. Blood lactic acid concentrations (mg %) of mature carp unexercised and during 16 hours after 15 minutes of forced exercise at 24°C—July, 1961 (mean, $\bar{x}$; range r; standard deviation, s; number of fish, n).

		Male	Female	Combined
Unexercised	$\bar{x}$	12	19	14
	r	9-17	19-19	9-19
	s	3.6	0.0	4.3
	n	6	2	8
0.0-0.4 hours after forced exercise	$\bar{x}$	34	26	29
	r	9-96	10-42	9-96
	s	41.5	11.5	27.1
	n	4	5	9
2.0-2.4 hours after forced exercise	$\bar{x}$	13	34	17
	r	3-33	9-58	3-58
	s	10.7	34.6	17.3
	n	8	2	10
4.0-4.4 hours after forced exercise	$\bar{x}$	17	24	19
	r	1-37	5-43	1-43
	s	15.1	26.9	17.2
	n	4	2	6
16.0-16.4 hours after forced exercise	$\bar{x}$	16		16
	r	1-39		1-39
	s	15.1		15.1
	n	9	0	9

Table 6. Analysis of variance of transformed blood lactic acid data from mature carp unexercised and during 16 hours after 15 minutes of forced exercise at 1°C—February-March, 1961.

Source of variation	Degrees of freedom	Sum of squares	Mean square
Sexes	1	0.047830	0.047830
Treatments	4	1.148963	0.287241
Interaction	4	0.128993	0.032248
Individuals	38	1.387048	0.036501

$F_{Sexes} = \frac{0.047830}{0.036501} = 1.31$ $\quad F_{0.05(1,38)} = 4.10$

$F_{Treatments} = \frac{0.287241}{0.036501} = 7.87$ $\quad F_{0.05(4,38)} = 2.62$

Table 4. Blood lactic acid concentrations (mg %) of mature carp unexercised and during 16 hours after 15 minutes of forced exercise at 1°C—February-March, 1961 (mean, $\bar{x}$; range, r; standard deviation, s; number of fish, n).

		Male	Female	Combined
Unexercised	$\bar{x}$	10	8	9
	r	6-16	6-13	6-16
	s	3.4	3.1	3.2
	n	6	4	10
0.0-0.5 hours after forced exercise	$\bar{x}$	20	30	22
	r	11-26	26-34	11-34
	s	5.9	5.6	7.0
	n	7	2	9
2.0-2.5 hours after forced exercise	$\bar{x}$	15	26	23
	r	9-23	11-44	9-44
	s	7.1	11.7	11.5
	n	3	7	10
4.0-4.4 hours after forced exercise	$\bar{x}$	11	13	12
	r	5-16	5-18	5-18
	s	3.6	7.2	4.6
	n	7	3	10
16.0-16.4 hours after forced exercise	$\bar{x}$	14	12	13
	r	5-23	7-16	5-23
	s	7.4	4.5	6.3
	n	6	3	9

(Secondat and Diaz, 1942; Black, 1957a, b, c; Black et al., 1959, 1960, 1962; Heath and Pritchard, 1962). However, this may have been due to the choice of sampling periods in this study, i.e. the further increase may have occurred without being detected. The ranges (Table 4) indicated that this might have been the case.

Among the studies of blood lactic acid of carp in the unexercised condition and immediately following 15 minutes of forced exercise (Table 8), the differences in the means of the unexercised groups at different temperatures are slight. Carp exercised at 11.5°C (Black, 1955) showed an approximately six- to eightfold increase in mean blood lactic acid concentration, while the increase at 1°C was approximately twofold in this study. Although data obtained at 24°C are included in Table 8, the failure to detect significant differences among the means rendered them of little value for comparative purposes. Thus, it is not possible at this time to describe the effect of temperature on blood lactic acid in fishes, although an effect might be expected due to differences in degree of muscular activity and/or diffusion rate of lactic acid (Johnson et al., 1945) from muscle to blood at different temperatures. Black (1957a) found no significant difference between the mean blood lactic acid concentrations of

Table 3. Total numbers and average total lengths and weights (ranges in parentheses) of mature carp used in statistical analyses of blood lactic acid data from February-March and July experiments, 1961.

	February-March	July
Number of fish		
Male	29	31
Female	19	11
Total length, cm		
Male	48 (36-64)	43 (28-58)
Female	50 (43-64)	56 (45-62)
Weight, kg		
Male	1.39 (0.73-2.35)	1.10 (0.23-2.35)
Female	1.74 (0.88-3.09)	2.30 (1.08-3.35)

An analyses of variance for a factorial arrangement of subclasses with disproportionate numbers (Snedecor, pp. 382-83) was then calculated with the transformed data for both experiments (Tables 6 and 7). Significant differences were indicated only among the treatment means of the February-March experiment. All remaining effects in both experiments were nonsignificant. A multiple range test (Kramer, 1956) of the February-March treatment means (sexes combined) showed that those of the "unexercised," "4.0-4.4 hours after forced exercise" and "16.0-16.4 hours after forced exercise" groups did not differ significantly. The same result was obtained for the "0.0-0.5 hours after forced exercise" and "2.0-2.5 hours after forced exercise" groups. The former three means were significantly lower than the latter two.

DISCUSSION

The mean blood lactic acid concentration of mature carp in February-March (1°C) increased, from an unexercised value of 9 mg %, approximately twofold to a value of 22 mg % 0.0-0.5 hours after 15 minutes of forced exercise. The higher level was maintained for at least 2.0-2.5 hours after exercise, then returned to the unexercised level by 4.0-4.4 hours after exercise. No further increase in the mean was noted following forced exercise, as has been observed in other investigations

Table 2. Treatment, sex, maturity, total length and weight of carp excluded from statistical analyses of blood lactic acid data from February-March and July experiments, 1961.

Experiment and treatment	Sex and maturity	Total length cm	Weight kg	Blood lactic acid mg %
February-March				
0.0-0.5 hours after forced exercise	F, mature	71	5.24	20
16.0-16.4 hours after forced exercise	M, mature	53	1.96	error
July				
Unexercised	F, immature	31	0.37	29
	F, immature	25	0.25	17
0.0-0.4 hours after forced exercise	M, mature	41	0.74	error
4.0-4.4 hours after forced exercise	F, immature	26	0.34	14
	F, mature	80	6.63	189
	F, mature	78	5.84	206
	F, immature	29	0.31	error
16.0-16.4 hours after forced exercise	F, immature	30	0.40	35

The remaining blood lactic acid data were considered with regard to sexes separated and sexes combined (Tables 4 and 5). In both cases, an increase in variability among individual fish with increase in the mean blood lactic acid concentration of a group of fish suggested heterogeneity of variance. Bartlett's test (Snedecor, 1956) showed that the treatment variances (sexes combined) of the February-March experiment and the treatment variances and subclass variances (sexes separated) of the July experiment were significantly different. The differences among the subclass variances of the February-March experiment approached significance ($0.10 < P < 0.25$). Thus, heterogeneity of variance was present and was not attributable to a possible sex difference in blood lactic acid.

Because the standard deviation was approximately proportional to the mean (Figures 1 and 2), i.e. the coefficient of variation was relatively constant, a logarithmic transformation seemed appropriate (Snedecor, 1956) to meet the assumption of homogeneity of variance for the analysis of variance. The data were transformed as follows:

$$X = \log_{10} (\text{mg \%}) \text{ blood lactic acid})$$

Table 1. Time sequence of experimental treatments, including time lapse (in parentheses) during each period of blood-sampling of 10 carp in February-March and July, 1961.

Treatments	February 28-March 1	July 21-22
Unexercised	10:18-10:52 am (34 minutes)	8:26-8:52 am (26 minutes)
0.0-0.4 or 0.5 hours after forced exercise	1:20-1:48 pm (28 minutes)	9:29-9:53 am (24 minutes)
2.0-2.4 or 2.5 hours after forced exercise	3:18-3:46 pm (28 minutes)	11:30-11:54 am (24 minutes)
4.0-4.4 hours after forced exercise	5:22-5:42 pm (20 minutes)	1:30-1:56 pm (26 minutes)
16.0-16.4 hours after forced exercise	5:21-5:39 am (18 minutes)	1:29-1:56 am (27 minutes)

Lactic acid analyses were conducted according to the modified colorimetric method of Barker and Summerson outlined in Hawk, Oser and Summerson (1954). A Bausch and Lomb "Spectronic 20" spectrophotometer and 3/4-inch optical test tubes were used. Lactic acid values were expressed in mg/100 ml of whole blood (mg %). The mean difference (sign ignored) between duplicate determinations on 18 filtrates (nine from each experiment) was 3 mg % (range 0-12 mg %) and served as a measure of the precision of the determinations.

RESULTS

Blood lactic acid data from nine carp were excluded from statistical analyses either because an error was made in lactic acid determination, the carp were immature, or they were considerably larger than the others (Table 2). The latter two reasons represented an attempt to present results for a more homogeneous sample of fish, but the remaining data are to this extent not random.

Information on size of the carp used in the analyses is presented in Table 3. In winter, the average total length of female carp was not significantly[6] different from that of the males, but the average weight of the females was significantly greater than that of the males. In summer, the females significantly exceeded the males in both average total length and average weight.

[6] Refers throughout this paper to the probability, $P = 0.05$.

County, in July, 1961, transferred to a crib in the lake, and held for one day prior to the experiment on July 21 and 22. Most of the carp were mature as shown by well developed gonads. The fish were not fed in either experiment.

In February-March, surface water temperature was near 1° C. The water was clear and the fish could easily be seen. They exhibited only limited voluntary activity. In July, surface water temperature was near 24°C. Water turbidity prevented the fish from being seen, but voluntary activity was thought to be greater than that observed in winter, because most of the fish were injured, usually about the head, apparently from attempts to escape the crib.

The experimental procedures were essentially similar in winter and summer. Each consisted of five treatments (Table 1) which initially included 10 fish.[4] The treatment sequence began with blood-sampling of the unexercised group. All remaining fish were simultaneously forced to swim for 15 minutes by chasing in the crib with a dip-net, after which blood samples were taken from groups of fish during four periods following exercise. Each fish was netted without selection as to size or sex and was sampled only once. This procedure differed from that used by Secondat and Diaz (1942) and Black (1955) in which cases the fish were apparently exercised individually, rather than simultaneously. This distinction is important because fish exercised in a group would probably respond less than those exercised individually (Shlaifer, 1938). In addition, sampling of a group of 10 fish required 18-34 minutes (Table 1) during which time further changes in blood lactic acid may have occurred.

Each fish was netted, stunned by a cranial blow and an incision was made along the belly, from the region slightly posterior to the pectoral fins, through the pectoral girdle to the gular region, to expose the heart. One ml (in February-March) or less[5] (average 0.3 ml, range 0.2-0.4 ml in July) of blood was extracted by cardiac puncture with a 2-ml Luer syringe into which heparin solution (1000 USP units ammonium heparinate per ml) had been introduced and the excess ejected. The syringe plunger was lubricated with mineral oil. Many syringes were used. The sample was immediately ejected into 9 ml of 10% trichloroacetic acid solution (w/v). This mixture was filtered within 2 hours and the filtrate, collected in a polyethelene vial, was placed on ice and later stored at -14°C until analyzed for lactic acid. The blood-sampling technique was similar to that employed by Parker and Black (1959) with the exception that an incision was made before extraction.

After a blood-sample was extracted the fish was weighed and measured (total length) and its gonads were examined to determine sex and maturity. The times taken to process each 10-fish group are presented in Table 1. The average time lapse between netting and completion of blood-sampling was 2.5 minutes (range 0.9-6.5 minutes) in winter and 1.1 minutes (range 0.2-2.5 minutes) in summer.

[4] The data from some fish were later excluded from statistical analyses for reasons to be discussed.

[5] Smaller samples were taken in an attempt to decrease sampling time. The possible increased error due to smaller blood-sample volumes was not considered to be serious.

INTRODUCTION

The importance of the study of lactic acid in fishes has been shown by its role in several interrelated phenomena including mortalities following hyperactivity (Black, 1958a; Parker and Black, 1959; Parker, Black and Larkin, 1959), rigor mortis (Amlacher, 1961) and carbohydrate metabolism (Black, 1958b; Gumbmann, Brown and Tappel, 1958; Black, Robertson and Parker, 1961).

Von Buddenbrock (1938) recognized that lactic acid accumulates in the blood of fishes following muscular activity. Changes in blood lactic acid related to time during and/or following forced exercise have been investigated in several fish species (Secondat and Diaz, 1942; Black, 1955, 1957a, b, c; Nakatani, 1957; Black et al., 1959, 1960, 1961, 1962; Miller, Sinclair and Hochachka, 1959; Heath and Pritchard, 1962). Such changes have also been studied following handling and transportation (Black, 1956; Black and Barrett, 1957), transportation and stream planting (Miller, 1958; Miller and Miller, 1962), capture by trolling (Parker and Black, 1959; Parker, Black and Larkin, 1959), hooking and tagging (Barrett and Connor, 1962) and air exposure (Leivestad, Andersen and Scholander, 1957; Scholander, Bradstreet and Garey, 1962). Denyes and Joseph (1956) considered the effects of size and temperature on blood lactic acid in unexercised fish. Among these studies, only two were conducted on carp, Cyprinus carpio L. Secondat and Diaz (1942) and Black (1955) determined blood lactic acid concentrations of unexercised carp and the latter author included data from carp exercised for 15 minutes.

The purpose of the present investigation was to determine the effect of forced muscular exercise on the blood lactic acid concentration of mature carp in winter and summer and to include observations on changes during the post-exercise recovery period.

MATERIALS AND METHODS

Two collections of carp were made. The first was seined from Lower Gar Lake, Dickinson County, Iowa, in February, 1961, transported to a crib[3] in a hatchery pond near the Spirit Lake Biology Station, Dickinson County, and held for 19 days prior to the experiment on February 28 and March 1. The second was seined from East Okoboji Lake, Dickinson

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[3] A wooden fish-holding box sunk in the water. The one used in February-March was approximately 6x6x6 ft; in July, 6x12x4 ft (deep).

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BLOOD LACTIC ACID CONCENTRATION OF UNEXERCISED AND EXERCISED MATURE CARP IN WINTER AND SUMMER[1]

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ABSTRACT. Blood lactic acid concentrations of mature carp in the unexercised condition and during 16 hours after 15 minutes of forced exercise were determined in winter, February-March, 1961 (1°C) and in summer, July, 1961 (24°C). In February-March, the mean blood lactic acid concentration increased, from an unexercised value of 9 mg/100 ml of whole blood (mg %) approximately twofold to a value of 22 mg % immediately after forced exercise. This higher level was maintained for at least 2.5 hours after exercise then returned to the unexercised level by 4.4 hours after exercise. No difference in mean blood lactic acid concentration was detected between male and female fish. In July, variability in blood lactic acid among individual fish was greater than in February-March, and no significant differences in mean blood lactic acid values were detected among unexercised and exercised groups or between sexes. The highest individual blood lactic acid levels were observed in July. These results may have represented an effect of higher temperature, but may also have been the result of an insufficient holding period prior to the experiment and a greater variability in voluntary (nonexperimental) and/or experimental activity among individual fish. In both studies, variability in blood lactic acid concentration among individual fish increased with increase in the mean. Because the standard deviation was approximately proportional to the mean, a logarithmic transformation was used.

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$$H_2(C) = \frac{B_2C^2 T_S}{(1 + B_1C + B_2C^2 + B_3C^3)} \qquad (18)$$

If the proper order in chloride has been chosen the resulting plot will be a straight line with a nonnegative intercept. It will be seen from Figs. 2 and 3 that a reasonable plot is obtained for n = 4 but not when n is chosen to be 3. The intercept along with the slope indicates that k' is about 0.32 L/M·min. and D is about 0.14 L/M by least squares treatment.

A determination for ferrocene itself in 1.68 N T_C and 0.155 M T_S gave an S(i) = 0.069 $min.^{-1}$. By comparing the rate constant for a particular dimethyl ferrocene determination with those of ethyl ferrocene and ferrocene for comparable chloride and stannous ion concentrations, a ratio of 1:(3-8):17 is obtained. This variation probably reflects the ease of formation of the $\overline{Fe}Cl$ complex in each case. Rabinowitch and Stockmayer (15) report $K_1^f = 3.8$ and $K_1^f K_2^f = 4.94$ for the ferric chloride complexes. Although these constants have not been evaluated for ferrocene or its derivatives they would be expected to be much lower. A comparison between ferric ion and ferricinium ion reduction rates at C = .476 M gives approximately a ratio of 1:9,000 (14), thus indicating that the electron transfer through the aromatic rings must be nonexistent or negligible and that the reaction occurs much more readily by way of the chloride bridge. The reduction in rate in going from ferricinium to dimethyl ferricinium ions indicates the molecule is still in its sandwich form and has not opened up on oxidation.

The data for ethyl ferrocene indicates a rate intermediate between the ferrocene and dimethyl ferrocene rates with an order of four in chloride but the sample was considered too impure to give quantitative data. Nevertheless, both the reaction rate and iron-chloride complexing constants are comparable to those of dimethyl ferrocene (k' is about 1.0 L/M·min. and D is about 0.5 L/M by least squares treatment).

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Table III. Data for reduction of ethyl ferrocene with stannous chlorides.

T_C M/L	T_S M/L	C M/L	$S(i)x10^3$ min^{-1}	Log Q(C)
2.47	0.156	2.08	42	2.167
1.80	.176	1.39	32	1.580
3.75	.0705	3.55	31.5	3.987
2.58	.0705	2.40	22.3	2.394
		Five weeks later		
1.68	.155		11.5	

Table IV. Data calculated for ethyl ferrocene (Eq. 17).

1/C	$H_1(C)$	$\frac{H_1(C)}{S(i)}$
0.481	.154	3.66
.719	.176	5.32
.282	.070	2.22
.417	.070	3.14

Table I. Data for reduction of dimethyl ferrocene with stannous chlorides.

T_C M	T_S M	C M	$S(i)\times10^3$ min^{-1}	Log Q(C)
3.02	0.184	2.54	9.25	1.731
2.89	.18	2.43	8.68	1.591
3.01	.18	2.53	9.14	1.659
2.66	.18	2.21	7.98	1.450
3.01	.167	2.53	8.68	1.668
2.47	.168	2.04	6.6	1.312
2.00	.168	1.63	5.06	0.957
1.235	.169	0.88	3.15	0.037
1.83	.169	1.44	4.63	0.863
5.17	.169	4.69	13.1	2.553
2.71	.160	2.29	6.93	1.480
1.87	.160	1.49	4.08	0.792
2.07	.156	1.69	4.48	0.974
2.13	.078	1.94	2.67	1.196
2.98	.078	2.78	3.96	1.764
2.38	.176	1.95	6.45	1.233
3.75	.0705	3.55	4.01	2.093

Table II. Data calculated for dimethyl ferrocene (Eq. 17).

$1/C$ M^{-1}	$H_1(C)$ M/L	$\frac{H_1(C)}{S(i)}$	$\frac{H_2(C)}{S(i)}$
.394	0.182	19.6	7.74
.411	.178	20.5	
.395	.178	19.5	
.452	.177	22.2	
.395	.165	19.0	
.490	.166	25.1	
.613	.165	32.6	
1.136	.168	53.5	60.8
.694	.166	35.4	
.213	.168	12.8	2.73
.437	.158	22.8	9.95
.671	.150	39.1	26.3
.591	.156	34.7	20.6
.515	.077	28.8	14.8
.360	.077	19.5	7.01
.513	.173	26.9	13.8
.282	.070	17.4	4.91

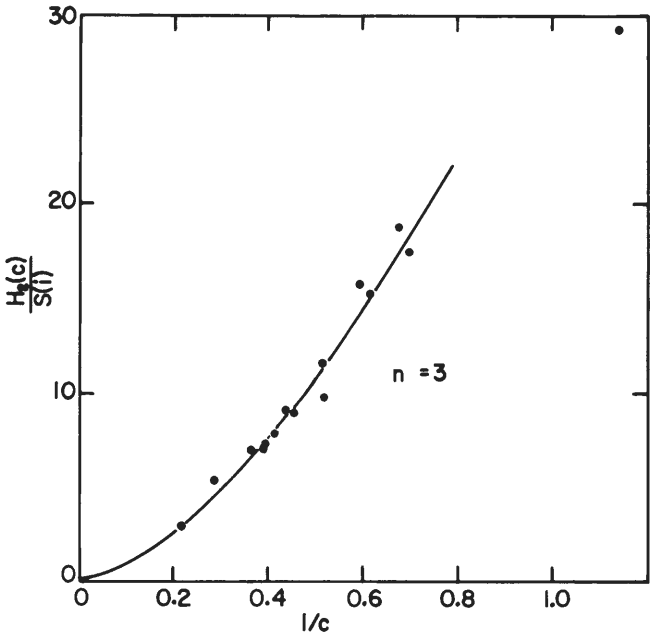

Figure 3. Dimethyl ferricinium reduction rate as a function of chloride conc. (Eq. 18).

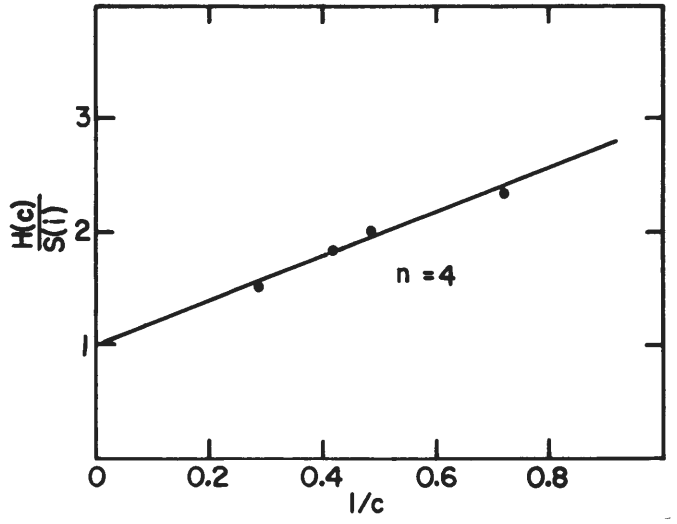

Figure 4. Ethyl ferricinium reduction rate as a function of chloride conc. (Eq. 17).

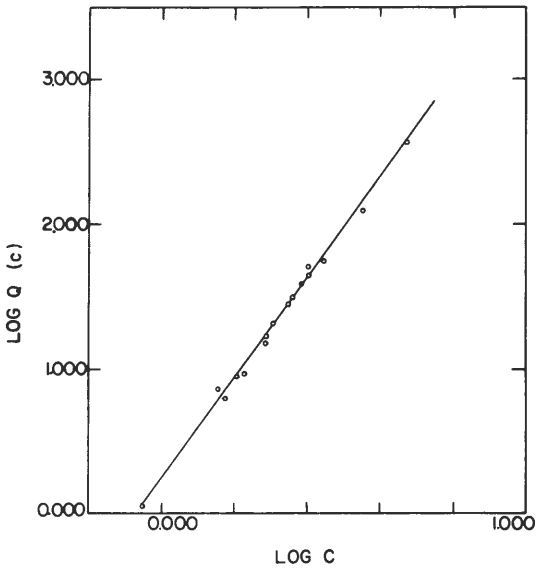

Figure 1. Dimethyl ferricinium reduction rate as a function of chloride conc. (Eq. 10).

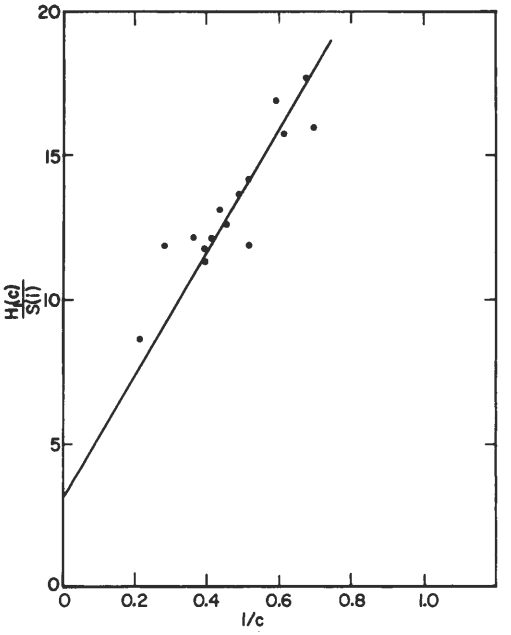

Figure 2. Dimethyl ferricinium reduction rate as a function of chloride conc. (Eq. 17).

When such a plot was made for dimethyl ferrocene [Bis(methylcyclopentadienyl)iron] the slope of the line appeared to be about 3.5 and K_2 about 1.7 min.$^{-1}$. This suggested that the order in chloride would be 3 or 4 but that a complex of chloride with the oxidant was involved in the reaction. Therefore the rate expression was rewritten to include an equilibrium constant for such a complex:

$$\frac{-d\, T_{\overline{\underline{Fe}}}}{dt} = k' \, (SnCl_m^{+2-m}) \, (FeCl), \tag{11}$$

where $\overline{\underline{Fe}}$ represents dimethyl ferricinium ion and $T_{\overline{\underline{Fe}}}$ the total initial concentration of the ion and its chloride complex. If D is the formation constant and M is 3, Eq. (11) can be developed as follows:

$$-dT_{\overline{\underline{Fe}}} = \frac{k' \, T_S \, B_3 \, C^3}{(1 + B_1C + B_2C^2 + B_3C^3)} \cdot \frac{D\, C\, T_{\overline{\underline{Fe}}}}{(1 + DC)} \tag{12}$$

Let

$$H_1(C) = \frac{T_S \, B_3 \, C^3}{(1 + B_1C + B_2C^2 + B_3C^3)} \tag{13}$$

then

$$\frac{-dT_{\overline{\underline{Fe}}}}{T_{\overline{\underline{Fe}}}} = \frac{k' \, H_1(C) DC}{1 + DC} \, dt \tag{14}$$

$$\ln T_{\overline{\underline{Fe}}} = \frac{k' \, H_1(C) \, DC \, t}{1 + DC} + f(t) \, . \tag{15}$$

When the normal first order plots of the individual determinations were made, straight lines were obtained, the slopes, S(i), of which should be equal to the slope of Eq. (15) at the particular stannous and chloride concentration of the run:

$$S(i) = \frac{k' \, H_1(C) \, DC}{1 + DC} \, . \tag{16a}$$

From this a plot can be made to evaluate k' and D,

$$\frac{S(i)}{H_1(C)} = \frac{DCk'}{1 + DC} \tag{16b}$$

$$\frac{H_1(C)}{S(i)} = \frac{1}{k'DC} + \frac{1}{k'} \, . \tag{17}$$

It can be seen from Eq. (17) that if $H_1(C)/S(i)$ is plotted against 1/C the intercept should be 1/k' and the slope should be 1/k'D (Figs. 2 and 4). In this development m will be equal to one less than n of the previous evaluation. If n should be 3 (m = 2) an equation similar to (13) will result with

and chloride concentrations were considered constant during the course of the reaction and the rate expression used was

$$\frac{-d(ox)}{dt} = k_1(ox). \tag{1}$$

The constant k_1 is a function of stannous and chloride ion, C, concentrations. If k_1 is considered dependent in the following way,

$$k_1 = k_2 (SnCl_n^{+2-n}) \tag{2}$$

$$= k_2 \, Bn(Sn^{+2})C^n, \tag{3}$$

the number n is the apparent order in chloride.

If total stannous concentration is T_S and total chloride concentration is T_C, the following expressions can be used

$$T_S = (Sn^{+2}) + (SnCl^+) + (SnCl_2) + (SnCl_3^-) \tag{4a}$$

$$T_S = (Sn^{+2}) (1 + B_1C + B_2C^2 + B_3C^3) \tag{4b}$$

$$T_C = (SnCl^+) + 2 (SnCl_2) + 3 (SnCl_3^-) + C \tag{5a}$$

$$T_C = (Sn^{+2}) (B_1C + 2B_2C^2 + 3B_3C^3) + C \tag{5b}$$

$$C = \frac{T_C}{1 + T_S R(C)} \tag{6}$$

where

$$R(C) = \frac{B_1 + 2B_2C + 3B_3C^2}{1 + B_1C + B_2C^2 + B_3C^3} \tag{7}$$

Equations (4b) and (5a) are derived from the equilibrium expressions for the three stannous complexes, which allow the concentrations of any complex to be expressed in terms of the concentrations of uncomplexed stannous ion and the chloride ion to the degree in which it depends, i.e.

$$(SnCl_3^-) = B_3(Sn^{+2})C^3 .$$

Equation (6) can then be solved numerically for C by successive approximations. Equation (3) can be further developed:

$$k_1 = \frac{K_2 \, T_S \, C^n}{(1 + B_1C + B_2C^2 + B_3C^3)} \quad ; \quad K_2 = k_2B_n \tag{8}$$

$$\frac{k_1(1 + B_1C + B_2C^2 + B_3C^3)}{T_S} = K_2 \, C^n = Q(C) \tag{9}$$

$$\log Q(C) = \log K_2 + n \log C . \tag{10}$$

Thus if log Q(C) is plotted against log C the slope of the resulting line should give the order in chloride ion, n (Fig. 1).

is absent, then the term $\left(\partial \ln m_i / \partial m_{BX}\right)_{T,p,m_k} dm_{BX}$ will be zero. It will be assumed in this treatment that binding or complexing between polymer units and between polymer and salt is absent. The term $\left(\partial \ln \gamma_{N,i} / \partial m_{BX}\right)_{T,p,m_k} dm_{BX}$ expresses the influence of the salt BX on the nonelectrolyte activity coefficient $\gamma_{N,i}$. The relative magnitude of this term is difficult to estimate. If, for example, the salt concentration is decreased, then the polymer should swell due to the repulsion of like charges within the polymer. This swelling may increase the nonelectrostatic interaction between polymer units. However, the change in concentration of the salt BX will be small from one radius to another in the ultracentrifuge cell unless the molecular weight of the salt is close to that of the polyelectrolyte. Therefore, when the ratio (M_{BX}/M_{PX_Z}) is small, one would expect this term to be negligible. The quantity dW_i contains similar interaction terms. Here, however, any change in the concentration of the salt BX may appreciably influence the cross-coefficient in the electrostatic term dW_i.

Employing the above assumption that complexing or binding is absent, then equation 1 becomes:

$$2A_i r dr = d \ln m_i + \left(\frac{1}{RT}\right) dW_i + \left(\frac{1}{RT}\right) dW_{N,i} \tag{2.0}$$

where

$$\left(\frac{1}{RT}\right) dW_{N,i} = \sum_{\substack{j=1 \\ j \neq BX}}^{q} \left(\frac{\partial \ln \gamma_{N,i}}{\partial m_j}\right)_{T,p,m_k} dm_j + \left(\frac{\partial \ln \gamma_{N,i}}{\partial m_{BX}}\right)_{T,p,m_k} dm_{BX} \tag{2.1}$$

and

$$\left(\frac{1}{RT}\right) dW_i = \sum_{\substack{j=1 \\ j \neq BX}}^{q} \left(\frac{\partial \ln \gamma_{d,i}}{\partial m_j}\right)_{T,p,m_k} dm_j + \left(\frac{\partial \ln \gamma_{d,i}}{\partial m_{BX}}\right)_{T,p,m_k} dm_{BX} + \sum_{\substack{j=1 \\ j \neq BX}}^{q} \left(\frac{\partial \ln \gamma_{\xi,i}}{\partial m_j}\right)_{T,p,m_k} dm_j + \left(\frac{\partial \ln \gamma_{\xi,i}}{\partial m_{BX}}\right)_{T,p,m_k} dm_{BX} \tag{2.2}$$

where $i = P_i X_{Z_i}$.

Equation 1 can also be applied to the salt BX by substituting i = BX instead of $i = P_i X_{Z_i}$. If it is again assumed that binding or complexing between polymer and salt is absent, then the term $\sum^{q} \left(\partial \ln m_i / \partial m_j\right) dm_j$ of equation 1 is zero when i = BX and $j = P_j X_{Z_j}$. Thus equation 1 becomes for the salt BX at the radius r:

$$2A_{BX} r dr = d \ln m_{BX} + \left(\frac{1}{RT}\right) dW_{BX} + \left(\frac{1}{RT}\right) dW_{N,BX} \tag{3.0}$$

where

$$\left(\frac{1}{RT}\right) dW_{N,BX} = \sum_{\substack{j=1 \\ j \neq BX}}^{q} \left(\frac{\partial \ln \gamma_{N,BX}}{\partial m_j}\right)_{T,p,m_k} dm_j + \left(\frac{\partial \ln \gamma_{N,BX}}{\partial m_{BX}}\right)_{T,p,m_k} dm_{BX} \tag{3.1}$$

and

$$\left(\frac{1}{RT}\right) dW_{BX} = \sum_{\substack{j=1 \\ j \neq BX}}^{q} \left(\frac{\partial \ln \gamma_{d,BX}}{\partial m_j}\right)_{T,p,m_k} dm_j + \left(\frac{\partial \ln \gamma_{d,BX}}{\partial m_{BX}}\right)_{T,p,m_k} dm_{BX} + \sum_{\substack{j=1 \\ j \neq BX}}^{q} \left(\frac{\partial \ln \gamma_{\xi,BX}}{\partial m_j}\right)_{T,p,m_k} dm_j + \left(\frac{\partial \ln \gamma_{\xi,BX}}{\partial m_{BX}}\right)_{T,p,m_k} dm_{BX} \tag{3.2}$$

Summation of the activity coefficient terms $\gamma_{n,i}$, $\gamma_{E,i}$, and $\gamma_{d,j}$: The summation terms in equations 2.1, 2.2, 3.1 and 3.2 can be treated in the same manner as given in the calculation of Kegeles et al. (27). If it is assumed that molalities equal molarities, i.e., $m_j = (C_j/M_j) = (C_{P_j} X_{Z_j}/M_{P_j X_{Z_j}})$, then the summation terms become at the radius r:

$$\sum_{\substack{j=1 \\ j \neq BX}}^{q} \left(\frac{\partial \ln \gamma_i}{\partial m_j}\right)_{T,p,m_k} dm_j = \sum_{\substack{j=1 \\ j \neq BX}}^{q} \left(\frac{\partial \ln \gamma_i}{\partial C_j}\right)_{T,p,C_k} dC_j \qquad (4.0)$$

where i equals (N, $P_i X_{Z_i}$), (d, $P_i X_{Z_i}$) or (E, $P_i X_{Z_i}$). The term d C_j can be defined as

$$dC_j = \left[\frac{(1-\bar{V}_j\rho)\omega^2}{RT}\right](M_j C_j)(r\,dr) \qquad (4.1)$$

by assuming the C^2 and higher terms in the concentration dependency of the molecular weight are negligible. Substitution of equation 4.1 into equation 4.0 and multiplying by $\sum_{j=1}^{q} M_j C_j / \sum_{j=1}^{q} M_j C_j$ or its equivalent $\bar{M}_{PX_Z W,r} C_{PX_Z,r} / \sum_{j=1}^{q} M_j C_j$ gives:

$$\sum_{\substack{j=1 \\ j \neq BX}}^{q} \left(\frac{\partial \ln \gamma_i}{\partial m_j}\right)_{T,p,m_k} dm_j = \langle k_i \rangle_Z \left[\bar{M}_{PX_Z W,r} C_{PX_Z,r}\right] \left[\frac{(1-\bar{V}\rho)\omega^2}{RT}\right](r\,dr) \qquad (4.2)$$

The term $(\bar{M}_{PX_Z W,r} C_{PX_Z,r}\, r\,dr)(1-\bar{V}\rho)(\omega^2/RT)$ is equal to d $C_{PX_Z,r}$ or d $C_{p,r}$ neglecting concentration dependence. Hence equation 4.2 becomes:

$$\sum_{\substack{j=1 \\ j \neq BX}}^{q} \left(\frac{\partial \ln \gamma_i}{\partial m_j}\right)_{T,p,m_k} dm_j = \langle k_i \rangle_Z\, dC_{p,r} \qquad (4.3)$$

where

$$\langle k_i \rangle_Z = \frac{\sum_{\substack{j=1 \\ j \neq BX}}^{q} \left(\frac{\partial \ln \gamma_i}{\partial C_j}\right)_{T,p,C_k} (M_j C_j)}{\sum_{\substack{j=1 \\ j \neq BX}}^{q} (M_j C_j)} \qquad (4.4)$$

Consequently, the expressions $\langle k_{N,i} \rangle_Z\, dC_{p,r}$, $\langle k_{d,i} \rangle_Z\, dC_{p,r}$ and $\langle k_{E,i} \rangle_Z\, dC_{p,r}$ can be substituted for their respective summation terms in equations 2.1, 2.2, 3.1 and 3.2. Here i = $P_i X_{Z_i}$ for equations 2.1 and 2.2 and i = BX for equations 3.1 and 3.2.

Treatment of a variable charge Z_i in a specific i^{th} molecular species. For the complete dissociation of the low and high molecular weight polyelectrolytes $P_i X_{Z_i}$ and BX equations 2.0 and 3.0 become:

$$d \ln m_{P,i} + Z_{P,i}\, d \ln m_X + \ln m_X\, dZ_{P,i} + (1/RT)\left[dW_{P_i X_{Z_i}} + dW_{N,P_i X_{Z_i}}\right] = 2A_{P_i X_{Z_i}}\, r\,dr \qquad (5.0)$$

and

$$d \ln m_B + d \ln m_X + \left(\frac{1}{RT}\right)\left[dW_{BX} + dW_{N,BX}\right] = 2A_{BX}\, r\,dr \qquad (5.1)$$

since $a_{P_iX_{Z_i}} = m_{P_i}^{Z_i} m_X^{Z_i} \gamma_{N,P_i} \gamma_{N,X}$ and $a_{BX} = m_B m_X \gamma_{N,B} \gamma_{N,X}$. Multiplying equation 5.1 by $Z_{P,i}$ and subtracting the result from equation 5.0 gives at the radius r:

$$d \ln m_{P,i} + \ln m_X \, dZ_{P,i} - Z_{P,i} \, d \ln m_B + \left(\frac{1}{RT}\right)\left[dW_{N,D,i} + dW_{D,i}\right] = 2A_{D,i} r dr \tag{6.0}$$

where

$$A_{D,i} = A_{PX_{Z_i}} - Z_{P,i} A_{BX} \tag{6.1}$$

$$dW_{D,i} = dW_{PiX_{Z_i}} - Z_{P,i} dW_{BX} \tag{6.2}$$

$$dW_{N,D,i} = dW_{N,PiX_{Z_i}} - Z_{P,i} dW_{N,BX} \tag{6.3}$$

The quantities in equations 6.2 and 6.3 are defined in equations 2.1, 2.2, 3.1, 3.2, 4.3 and 4.4. When this subtraction is done for each i^{th} species, there will be a total of i equations such as equation 6.0. However, if the electrostatic charge on a polymer species varies, the number of such equations will increase. The charge $Z_{P,i}$ may vary even though the molecular weight remains constant, i.e., $Z_{P,i}$ may vary for a given i^{th} species. The quantity $Z_{P,i}$ therefore represents an average charge for all of the i^{th} species. If the final equation is expressed in terms of moles, then $dZ_{P,i}$, $Z_{P,i}$, $dW_{N,D,i}$ and $dW_{D,i}$ will be number-average values. If, however, the concentration of the polymer in the final equation is expressed in terms of weight per unit volume, then the average will be a weight-average value. This result comes from the $d \ln m_{P,i}$ term of equation 6.0, which can be expressed by the quantity $(d m_{P,i}/m_{P,i})$ or $(d C_{P,j}/C_{P,j})$. For example, consider that the i^{th} molecular species can exist in n forms having n different net charges $Z_{P,i,j}$. Assuming that molalities equal molarities, i.e., $m_{P,i,j} = (C_{P,i,j}/M_{P,i})$, multiplying equation 6.0 by $(C_{P,i,j}/C_{P,i,j})$ and summing over all j species of the i^{th} component (from j = 1 to j = n), then equation 6.0 becomes:

$$\left[\frac{d\sum_{j=1}^{n} C_{P,i,j}}{\sum_{j=1}^{n} C_{P,i,j}}\right] + (\ln m_X)\left[\frac{\sum_{j=1}^{n}(dZ_{P,i,j})C_{P,i,j}}{\sum_{j=1}^{n} C_{P,i,j}}\right] - (d \ln m_B)\left[\frac{\sum_{j=1}^{n} Z_{P,i,j} C_{P,i,j}}{\sum_{j=1}^{n} C_{P,i,j}}\right] + \left(\frac{1}{RT}\right)\frac{\sum_{j=1}^{n}\left[dW_{N,D,i,j} + dW_{D,i,j}\right]C_{P,i,j}}{\sum_{j=1}^{n} C_{P,i,j}} = 2A_{D,i} r dr \tag{6.4}$$

or

$$\frac{dC_{P,i}}{C_{P,i}} + (\ln m_X) dZ_{P,i,W_j} - Z_{P,i,W_j} d \ln m_B + \left(\frac{1}{RT}\right)\left[dW_{N,D,i,W_j} + dW_{D,i,W_j}\right] = 2A_{D,i} r dr \tag{6.5}$$

Here the subscript W_j signifies that the term is the weight-average value of all j^{th} species of an i^{th} type molecule. Since equation 6.5 will be used, then the terms that are influenced by a variation in the charge for a given i^{th} species will be weight-average values.

Simplification of the B coefficient. Equation 6.4 can be readily summed over all q components of the $P_iX_{Z_i}$ polyelectrolyte to give the weight-average value of $A_{D,i,r}$. However, in order to simplify the equations for the determination of other weighted average molecular weights, it will be assumed that the ratio of charge to mass $(Z_{P,i}/M_{P,i})$ remains constant. In a few cases this constancy may not be true. For

example, the electrostatic interaction of unlike charges—such as that in α-amino acids or in the aggregation of some protein molecules (11)—or the electrostatic interaction of like charges may change the total charge by changing the pK of the ionizing groups. In addition, it will be assumed that the terms dW_{N,D,i,W_i} and dW_{D,i,W_i} are proportional to the molecular weight of the polymer, i.e., $(dW_{N,D,i,W_i}/M_{D,i})$ and $(dW_{D,i,W_i}/M_{D,i})$ are constant.

The molality of X^- in eq. 6.5 is $m_x = m_B + Z_P m_P$ where Z_P is the average charge and m_P is the total molal concentration of the polymer. Assuming that $Z_{P,i}$, $dW_{N,D,i}$ and $dW_{D,i}$ are proportional to $M_{D,i}$, then at the radius r equation 6.5 becomes:

$$2A_{D,i,r}C_{P,i,r} = \left(\frac{dC_{P,i}}{rdr}\right)_r \Big/ \left[1 + \left(\frac{B_D^\dagger}{dC_{P,i}}\right) M_{D,i} C_{P,i}\right]_r \qquad (7.0)$$

where $B_{D,r}^\dagger = B_{D,r}\, dC_{P,r}$ and where

$$B_{D,r} = -\left(\frac{Z'_P}{M'_D}\right)_r \left(\frac{d \ln C_B}{dC_P}\right)_r + \left(\frac{1}{RT}\right)\left(\frac{dW'_{N,D}}{M'dC_P}\right)_r + \left(\frac{1}{RT}\right)\left(\frac{dW'_D}{M'_D dC_P}\right)_r + \left[\ln\left(\frac{C_B}{M_B} + \frac{Z'_P C_P}{M'_P}\right)\right]_r \left[\frac{d(Z'_P/M'_D)}{dC_P}\right]_r \qquad (7.1)$$

In equation 7.1 the prime designates the subunit or monomer unit of the polymer. By assuming that the ratio of charge to mass $(Z_{P,i}/M_{D,i})$, that the ratio of the nonelectrostatic coefficients to mass $(dW_{N,D,i}/M_{D,i})$ and that the ratio of the electrostatic coefficients to mass $(d W_{D,i}/M_{D,i})$ are the same for all molecular species, the $B_{D,i}$ coefficients of equation 7.0 becomes the same for all i^{th} species, i.e., $B_{D,i} = B_D$. In order to simplify the terminology, the W_j subscript of equation 6.5 has not been included in equations 7.0 and 7.1 and subsequent equations. In equation 7.0 the value of the $dW_{N,D}$ term as obtained from equations 6.3, 4.3, 3.1 and 2.1 is:

$$\left(\frac{1}{RT}\right)\left(\frac{dW'_{N,D}}{M'_D dC_P}\right) = \left[\left(\frac{\langle k'_{N,i}\rangle_I}{M'_D}\right) - \left(\frac{Z'_P}{M'_D}\right)\langle k_{N,BX}\rangle_I\right] + \left[\left(\frac{1}{M'_D}\right)\left(\frac{\partial \ln \gamma'_{N,I}}{\partial C_{BX}}\right)_{T,P,C_k} - \left(\frac{Z'_P}{M'_D}\right)\left(\frac{\partial \ln \gamma_{N,BX}}{\partial C_{BX}}\right)_{T,P,C_k}\right]\left(\frac{dC_{BX}}{dC_P}\right) \qquad (7.2)$$

where $i = PX_Z$ and where $\langle k_i\rangle_I$ is defined as given in equation 4.4. The dW'_D term can be expressed in the same manner by incorporating γ_d and γ_E for γ_N in equation 7.2.

Expressing M_i and $C_{P,i}$ in the B coefficient as $\bar{M}_W$ and C_P. The concentration coefficient in equation 7.0 can be expressed in terms of $\bar{M}_{D,W,r}C_{P,r}$ instead of $M_{D,i,r}C_{P,i,r}$. Using equation 7.0 for the definition of $M_{D,i,r}$ and $A_{D,i,r}$, then we obtain for the concentration coefficient term of equation 7.0:

$$B_D^\dagger (M_{D,i}\, C_{P,i}/dC_{P,i})_r = B_D^\dagger (1/rdr)\left[1 + \left(\frac{B^\dagger}{dC_{P,i}}\right) M_{D,i} C_{P,i}\right]_r \left[\frac{RT}{(1-\bar{V}_{D,i}\rho)\omega^2}\right] \qquad (8)$$

Here it should be noted that $(1/r\ d\ r)$ is not a function of the i^{th} species and, hence, can be converted to values dealing with either the total concentration C_P or the concentration of the i^{th} species $C_{P,i}$. If it is assumed that $(\bar{M}_{W,D}C_P/d\ C_P)_r$ has the same concentration dependence as

$(M_{D,i}C_{p,i}/d\ C_{p,i})_r$ in the B_D^+ coefficient of equation 7.0, then equation 7.0 becomes at the radius r:

$$2A_{D,i,r}\,C_{p,i,r} = \left(\frac{dC_{p,i}}{rdr}\right)_r \left\{1 + B_D\bar{M}_{w,D}C_p\right\}_r \tag{9}$$

This treatment differs from that given previously (3) where it was assumed that $(B_D^+/d\ C_{p,i})_r$ was constant for all species instead of $(B_D^+/d\ C_p)_r$. If the former is assumed, then the concentration coefficient is multiplied by $M_{D,i,r}C_{p,i,r}$ as in equation 7.0 instead of $\bar{M}_{w,D,r}C_{p,r}$ as in equation 9. However, it will be shown below that both methods give the same result for $\bar{M}_w$ and $\bar{M}_z$ as well as $\bar{M}_{w,r}$ and $\bar{M}_{z,r}$.

Expressions for $\bar{M}_{w,r}$ and $\bar{M}_{z,r}$

In terms of molecular weights, equation 9 becomes

$$M_{D,i}C_{p,i,r} = \left[\frac{RT}{(1-\bar{V}_{D,i}\rho)\omega^2}\right]\left(\frac{dC_{p,i}}{rdr}\right)_r\left[1 + B_D\bar{M}_{w,D}C_p\right]_r \tag{10}$$

where $M_{D,i}$ is related to $A_{D,i}$ by the definitions given in equations 1 and 6. Summing over all species and assuming that all partial specific volumes of the polymer molecules are the same, equation 10 can be put into the form:

$$\frac{1}{\bar{M}^{app}_{w,p,r}} = \left(\frac{1}{\bar{M}_{w,D,r}}\right) + B_{D,r}C_{p,r} \tag{11.0}$$

where $B_{D,r}$ is defined as in equation 7.1. Also

$$\bar{M}^{app}_{w,p,r} = \left[\frac{RT}{(1-\bar{V}_p\rho)\omega^2}\right]\left(\frac{1}{C_{p,r}}\right)\left(\frac{dC_{p,r}}{rdr}\right) \tag{11.1}$$

assuming $\bar{V}_p = \bar{V}_D$.

Equation 11.0 is similar to that obtained by Kegeles et al. (27) for a nonelectrolyte:

$$\frac{1}{\bar{M}^{app}_{w,p,r}} = \frac{1}{\bar{M}_{w,D,r}} + \left[\frac{\langle k_{N,i}\rangle_z}{\bar{M}_{w,D}}\right]_r C_{p,r} \tag{12}$$

where $\langle k_{N,i}\rangle_z = (\Sigma\langle k_{N,i}\rangle_z\ C_{p,i})/\Sigma C_{p,i}$ and where $\langle k_{N,i}\rangle_z$ is defined in equation 4.4. The resulting equation 12 can be obtained by summing equation 6.5 without assuming a constant (B_D'/M_D') value. The coefficient given in equation 12 can be compared to that given in equation 11.0. For a nonelectrolyte, the $B_{D,r}$ term of equation 11.0 becomes $\left[\langle k_{N,i}\rangle_z/M_D'\right]_r$ as compared to $\left[\langle k_{N,i}\rangle_z/\bar{M}_{w,D}\right]$ of equation 12.

Multiplying equation 10 by M_i, summing all components, substituting the value of $\bar{M}_{w,r}C_r$ as obtained from equation 11.0 and assuming that all C^2 terms are negligible as in developing equations 18 to 24 of the previous paper (10), then we find that equation 10 becomes:

$$\frac{1}{\bar{M}^{app}_{z,r}} = \frac{1}{\bar{M}_{D,z,r}} + 2B_{D,r}\left(\frac{\bar{M}_{D,w,r}}{\bar{M}_{D,z,r}}\right)C_r \tag{13.0}$$

where

$$M_{Z,r}^{app} = \left[\frac{RT}{(1-\bar{V}\rho)\omega^2}\right]\left[\frac{d\left(\frac{dC}{rdr}\right)}{dC}\right]_r \qquad (13.1)$$

and where $B_{D,r}$ is defined as in equation 7.1. The subscript p has been dropped in equation 13 and all subsequent equations since the concentrations now refer only to the total polymer PX_Z unless otherwise specified. From equations 11.0 and 13.0 the concentration coefficient for the Z-average molecular weight is $2(\bar{M}_{w,r}/\bar{M}_{z,r})$ times larger than that for the weight-average molecular weight. This result agrees with that obtained previously (10) where it was assumed that each i^{th} species has the concentration coefficient BM_iC_i as given in equation 7.0 and not $B\bar{M}_wC$ as used above.

Determination of $\bar{M}_w$ and $\bar{M}_z$

The same relationship as given for $\bar{M}_{w,D,r}$ and $\bar{M}_{z,D,r}$ will now be shown to be true for $\bar{M}_{w,D}$ and $\bar{M}_{z,D}$ using $B_D\bar{M}_{w,D,r}C_r$ as the concentration coefficient term. By employing equation 11.0 to obtain the values $(\bar{M}_{w,D,a}C_a)$ and $(\bar{M}_{w,D,b}C_b)$ where a and b are the meniscus and cell bottom, an equation for $\bar{M}_{D,Z}$ can be developed as shown previously (see equations 35 to 39 of reference 10). The result is

$$\frac{1}{\bar{M}_Z^{app}} = \frac{1}{\bar{M}_{D,Z}} + \left[\frac{(B_D\bar{M}_{D,W}C)_a + (B_D\bar{M}_{D,W}C)_b}{\bar{M}_{D,Z}}\right]\left(\frac{1}{\bar{M}_{D,Z}}\right) + 2B_{D,b}\left(\frac{\bar{M}_{D,W,b}}{\bar{M}_{D,Z}}\right)\left[\frac{hC_a+C_b}{2}\right] \qquad (14)$$

where $h = (B_D\bar{M}_{D,W})_a/(B_D\bar{M}_{D,W})_b$ and where a and b are the meniscus and cell bottom in the ultracentrifuge cell. If we assume that $(C_a + C_b)$ is essentially the same as $(hC_a + C_b)$, then equation 14 becomes

$$\frac{1}{\bar{M}_Z^{app}} = \left(\frac{1}{\bar{M}_{D,Z}}\right) + 2B_{D,b}\left(\frac{\bar{M}_{D,W,b}}{\bar{M}_{D,Z}}\right)\left(\frac{C_a+C_b}{2}\right) \qquad (15)$$

where

$$M_Z^{app} = \left[\frac{RT}{(1-\bar{V}\rho)\omega^2}\right]\left[\frac{\Delta\left(\frac{dC}{rdr}\right)}{\Delta C}\right]_{(a\ to\ b)}$$

The value of $\bar{M}_{D,w}$ can be obtained by the method of Van Holde and Baldwin (50), i.e., by substitution of the value of rC_idr as obtained from equation 10 into the expression:

$$\int_a^b rC_i dr = (C_i^\circ/2)(b^2-a^2)$$

Thus the process of integration and summation can be carried out as indicated:

$$\sum_i M_i\int_a^b rC_{i,r}dr = \left[\frac{RT}{(1-\bar{V}\rho)\omega^2}\right]\left\{\int_a^b d\left(\sum_i C_{i,r}\right) + \int_a^b B_{D,r}\bar{M}_{D,W,r}C_r d\left(\sum_i C_{i,r}\right)\right\} \qquad (16)$$

which yields:

$$\bar{M}_{D,w} = \left[\frac{2RT}{(1-\bar{V}\rho)\omega^2}\right]\left(\frac{1}{b^2-a^2}\right)\left\{(C_b - C_a) + \int_a^b B_{D,r}\bar{M}_{D,w,r}C_r\,dC_r\right\} \tag{17}$$

The integral on the right of equation 17 may be assumed to be equal to $(1/2)\left\{(B_D\bar{M}_{D,w}C^2)_b - (B_D\bar{M}_{D,w}C^2)_a\right\}$, i.e., integrating by parts and assuming that the integral $-\int_a^b (C^2/2)\,d(B_{D,r}\bar{M}_{D,w,r})$ equals zero. Here $\bar{M}_{D,w,r}$ is defined as given in equation 11.0. This integral in turn may be approximated as $(1/2)(C_b - C_a)[(B_D\bar{M}_{D,w}C)_a + (B_D\bar{M}_{D,w}C)_b]$. If these assumptions are used, equation 17 becomes

$$\frac{1}{\bar{M}_w^{app}} = \left(\frac{1}{\bar{M}_{D,w}}\right) + \left[\frac{(B_D\bar{M}_{D,w}C)_a + (B_D\bar{M}_{D,w}C)_b}{2}\right] = \left(\frac{1}{\bar{M}_{D,w}}\right) + B_{D,b}\left(\frac{\bar{M}_{D,w,b}}{\bar{M}_{D,w}}\right)\left[\frac{hC_a + C_b}{2}\right] \tag{18}$$

where $h = (B_D\bar{M}_{D,w})_a/(B_D\bar{M}_{D,w})_b$. If $(C_a + C_b)$ is essentially the same as $(hC_a + C_b)$ then equation 18 can be approximated as

$$\frac{1}{\bar{M}_w^{app}} = \left(\frac{1}{\bar{M}_{D,w}}\right) + B_{D,b}\left(\frac{\bar{M}_{D,w,b}}{\bar{M}_{D,w}}\right)\left(\frac{C_a + C_b}{2}\right) \tag{19}$$

where

$$\bar{M}_w^{app} = \frac{(C_b - C_a)}{C^2(b^2-a^2)}\left[\frac{RT}{(1-\bar{V}\rho)\omega^2}\right]$$

These equations are in the same form as equation 33 and 34 of reference 10. For a homogeneous polymer ($\bar{M}_{D,w} = \bar{M}_{D,z}$) the concentration dependent terms of equations 15 and 19 are the same as those derived by Van Holde and Baldwin (50). In addition, when $C_a = 0$, then $\bar{M}_{n,b}^{app} = \bar{M}_w^{app}$, $\bar{M}_{w,b}^{app} = \bar{M}_Z^{app}$ and $\bar{M}_{Z,b}^{app} = \bar{M}_{(Z+1)}^{app}$ according to Yphantis (61). These relationships can be applied to equations 15 and 19.

Calculation of $\bar{M}_{n,r}$ and $\bar{M}_n$

The number-average molecular weight can be obtained by first rearranging equation 10 into the form:

$$C_{i,r}r\,dr = \left[\frac{RT}{(1-\bar{V}\rho)\omega^2}\right]\left(\frac{1}{\bar{M}_{D,i,r}}\right)\left[1 + B_{D,r}\bar{M}_{D,w,r}C_r\right]dC_{i,r} \tag{20}$$

Integration from the meniscus (r = a) to any point r in the cell plus summation over all i^{th} species gives:

$$\int_a^r C_r r\,dr = \left[\frac{RT}{(1-\bar{V}\rho)\omega^2}\right]\left\{\sum_i\left(\frac{C_{i,r}}{\bar{M}_{D,i}}\right) - \sum_i\left(\frac{C_{i,a}}{\bar{M}_{D,i}}\right) + \int_a^r B_{D,r}\bar{M}_{D,w,r}C_r\,d\left[\sum_i\left(\frac{C_{i,r}}{\bar{M}_{D,i}}\right)\right]\right\} \tag{21}$$

The value of $\sum_i(C_{i,r}/\bar{M}_{D,i})$ can be obtained from the definition of $\bar{M}_{D,n,r}$: $\bar{M}_{D,n,r} = C_r/\sum_i(C_{i,r}/\bar{M}_{D,i})$. Because the constant $\sum(C_{a,i}/\bar{M}_{D,i})$ must be estimated on the basis of additional assumptions, Yphantis (7) has proposed that the experimental run be made at high speeds where the meniscus concentration is zero. The concentration can be determined quite readily using schlieren optics. The integral to the right of equation 21 can be expressed as $\int_a^r B_{D,r}(\bar{M}_{D,w,r}\ \bar{M}_{D,n,r})(C_r/\bar{M}_{D,n,r})\,d(C_r/\bar{M}_{D,n,r})$. This integral has the same form as that given in equation 17. It can thus be assumed, as in equation 18, that

integration of this term yields:

$$\left(\frac{1}{2}\right)\left[\left(\frac{C_r}{\bar{M}_{D,n,r}}\right)-\left(\frac{C_a}{\bar{M}_{D,n,a}}\right)\right]\left[\left(B_D\bar{M}_{D,W}\bar{M}_{D,n}\right)_a\left(\frac{C}{\bar{M}_{D,n}}\right)_a+\left(B_D\bar{M}_{D,W}\bar{M}_{D,n}\right)_r\left(\frac{C}{\bar{M}_{D,n}}\right)_r\right]$$

If the concentration at the meniscus is zero ($C_a = 0$), then with the various assumptions given, equation 21 becomes:

$$\frac{1}{\bar{M}_{n,r}^{app}} = \left(\frac{1}{\bar{M}_{D,n,r}}\right) + \left(B_{D,r}/2\right)\left(\bar{M}_{D,W,r}/\bar{M}_{D,n,r}\right)C_r \qquad (22)$$

where

$$\bar{M}_{n,r}^{app} = \left[\frac{RT}{(1-\bar{V}\rho)\omega^2}\right] C_r \Big/ \int_a^r C_r\, r\, dr$$

When r = b then

$$\bar{M}_{D,n,b}^{app} = \bar{M}_{D,W}^{app} = \left[\frac{2RT}{(1-\bar{V}\rho)\omega^2}\right]\left[\frac{C_b}{C^\circ(b^2-a^2)}\right]$$

The ratios of the concentration dependence of a polymer as obtained from $\bar{M}_{D,n,r}$, $\bar{M}_{D,w,r}$ and $\bar{M}_{D,a,r}$ are $(1/2)\,(\bar{M}_{D,w,r}/\bar{M}_{D,n,r}):1:(2)\,(\bar{M}_{D,w,r}/\bar{M}_{D,z,r})$. If the special case $\bar{M}_{D,n,r}:\bar{M}_{D,z,r} = 1:2:4$ exists, then all coefficients are equal. Alternatively, if $\bar{M}_{D,n,r} = \bar{M}_{D,w,r}$, i.e., a homogeneous polymer, then the respective ratios of the coefficients will also be 1:2:4. In other words, the slope of a plot of $1/\bar{M}_{Z,r}^{app}$ versus C_r for a homogeneous polymer will be four times as great as the slope obtained in a plot of $1/\bar{M}_{n,r}^{app}$ versus C_r.

The value of the number-average molecular weight of the entire solute, if equation 22 is used for the definition of $\bar{M}_{D,n,r}$, is (47):

$$\bar{M}_{D,n} = \frac{\int_a^b C_r\, r\, dr}{\int_a^b \left(r C_r/\bar{M}_{D,n,r}\right) dr} = \frac{C^\circ(b^2-a^2)/2}{\left[\int_a^b \left(\frac{rC_r}{\bar{M}_{n,r}^{app}}\right) dr - \int_a^b \left(\frac{B_r}{2}\right)\left(\frac{\bar{M}_{D,W}}{\bar{M}_{D,n}}\right)_r C_r^2\, r\, dr\right]} \qquad (23)$$

The definition of $\bar{M}_{n,r}^{app}$ as given in equation 22 can be substituted into equation 23 and two assumptions can be made: first, $B_{D,r}(\bar{M}_{D,w}/\bar{M}_{D,n})_r$ remains constant from a to b and is equal to $B_b(\bar{M}_{D,w}/\bar{M}_{D,n})_b$ and, secondly, the integral $\int_a^b r C_r^2 dr = \left[(C_a + C_b)/2\right]\int_a^b r C_r dr$. Then equation 23 becomes at $C_a = 0$:

$$\frac{1}{\bar{M}_n^{app}} = \left(\frac{1}{\bar{M}_{D,n}}\right) + \left(\frac{B_{D,b}}{2}\right)\left(\frac{\bar{M}_{D,W,b}}{\bar{M}_{D,n,b}}\right)\left(\frac{C_b}{2}\right) = \left(\frac{1}{\bar{M}_{D,n}}\right) + \left(\frac{B_{D,b}}{2}\right)\left(\frac{\bar{M}_{D,z}}{\bar{M}_{D,W}}\right)\left(\frac{C_b}{2}\right) \qquad (24)$$

where

$$\bar{M}_n^{app} = \left[\frac{RT}{(1-\bar{V}\rho)\omega^2}\right]\left[\frac{C^\circ(b^2-a^2)}{2}\right]\Big/\int_a^b\left[\int_a^r C r dr\right] r dr$$

A comparison of equations 15, 19 and 24 at $C_a = 0$ shows that the ratio of the concentration dependence for the determination of $\bar{M}_{D,n}$, $\bar{M}_{D,w}$ and $\bar{M}_{D,z}$ is almost the same as that for $\bar{M}_{D,n,r}$, $\bar{M}_{D,w,r}$ and $\bar{M}_{D,z,r}$. The only difference is that the coefficient for $\bar{M}_{D,w}$ is either two times or $(\bar{M}_{D,z}\bar{M}_{D,n}/2\bar{M}_{D,w}^2)$ times as large as that for $\bar{M}_{D,n}$. This exception

may be due to the assumptions used in deriving equation 24, e.g., $B_D(\bar{M}_{D,w}/\bar{M}_{D,n}) = B_{D,b}(\bar{M}_{D,z}/\bar{M}_{D,w})$ remains constant at all values of r.

Extrapolated Molecular Weight

In a previous paper (10) it was shown that for a heterogeneous polymer the quantity $A_{2,i} = A_{P_iX_{Z_i}} - (Z_{P,i}/2)A_{BX}$ can be readily converted into expressions for $\bar{M}_w$ and $\bar{M}_z$ if it is assumed that the ratio of charge to mass remains constant. These results can be directly applied to the definition $A_{D,i} = A_{P_iX_{Z_i}} - Z_{P,i}A_{BX}$ since the only difference is the factor two. It was shown (13) that the expression $Z_{P,i}A_{BX}$ instead of $(Z_{P,i}/2)A_{BX}$ gives the correct extrapolated molecular weight. Therefore the relationship between the extrapolated and true molecular weights is:

$$\bar{M}_{D,w} = \bar{M}_{PX_{z,w}}\tau \tag{25}$$

and

$$\bar{M}_{D,z} = \bar{M}_{PX_{z,z}}\tau \tag{26}$$

where

$$\tau = \left[1 - M_{BX}\left(\frac{Z'_P}{M'_{PX_z}}\right)\left(\frac{1-\bar{V}_{BX}\rho}{1-\bar{V}_{PX_z}\rho}\right)\right]$$

It can also be shown that

$$\bar{M}_{D,n} = \bar{M}_{PX_{z,n}}\tau \tag{27}$$

Here $\bar{M}_{D,w}$, $\bar{M}_{D,z}$ and $\bar{M}_{D,n}$ are the extrapolated molecular weights obtained by plotting the reciprocal of the respective apparent molecular weight versus the concentration of the polymer, i.e., the approximate value $(C_a + C_b)/2$. The above relationships are also true for $\bar{M}_{D,n,r}$, $\bar{M}_{D,w,r}$ and $\bar{M}_{D,z,r}$.

Discussion

The expression for the concentration coefficient of the $i^{\underline{th}}$ species at the radius r is given in equation 7.1. By incorporating the molecular weight $M_{D,i}$ of the $i^{\underline{th}}$ species into this expression, a coefficient is obtained that in many cases should be approximately independent of the size of the molecular species. This $B_{D,r}$ coefficient contains terms for the activity coefficients due to nonelectrostatic interactions $\gamma_{N,i}$, electrostatic interactions $\gamma_{E,i}$ and dipole-dipole interactions $\gamma_{d,i}$. The $\gamma_{N,P_iX_{Z_i}}$, γ_E, $P_iX_{Z_i}$ and $\gamma_{D,P_iX_{Z_i}}$ are weighted-average values (equations 7.2 and 4.4). In addition to these coefficients, the $B_{D,r}$ coefficient contains the two terms $(-d \ln C_B)$ and $d(Z_P'/M_D')$, which are not weighted-averaged if one assumes that (Z_p'/M'_D) is independent of the size of the polymer molecule. That is, heterogeneity enters into these terms only in the $(Z_{P,i}/M_{D,i})$ ratio. If the ratio $(Z_{P,i}/M_{D,i})$ is not the same for all polymer species, then the weight-average value $(1/C_P)\Sigma(Z_{P,i}/M_{D,i})C_{P,i}$ must be used if the optical system relates the concentration on a weight per unit volume basis.

The equations 11.0, 13.0, 15, 19, 22 and 24 for $\bar{M}_{w,r}$, $\bar{M}_{z,r}$, $\bar{M}_z$, $\bar{M}_w$, $\bar{M}_{n,r}$ and $\bar{M}_n$, respectively, apply for an uncharged polymer as well as an electrostatically charged polymer. If the net charge on the polymer is zero, then the only remaining terms are the nonelectrostatic coefficient $\gamma_{N,i}$ and the dipole moment coefficient $\gamma_{d,i}$ as expressed in the dW term. By applying the excluded volume theory to osmotic pressure and light-scattering measurements, Flory (18, 19) has shown that the activity coefficient for uncharged polymers is dependent on the molecular weight and the heterogeneity of the polymer. Varadaiah and Rao (51) and others (4, 8) have examined the change in this coefficient with heterogeneity using light-scattering techniques. As pointed out by Flory and Krigbaum (19), for "normal" degrees of heterogeneity, the ratio of the B coefficient obtained from the weight-average molecular weight to that obtained from number-average values will not differ much from two. This ratio is changed appreciably from two for broad molecular size distributions (19). For charged polymers both the B coefficient itself and the ratio of the B coefficients as obtained from the $\bar{M}_n$, $\bar{M}_w$ and $\bar{M}_z$ ultracentrifugal calculations will depend on the heterogeneity of the sample even if charge effects or if nonelectrostatic effects are absent. Thus heterogeneity enters into the B coefficient without applying the work of Flory (18, 19) to the ultracentrifuge-equilibrium equations. However, it should be noted that Eisenberg and Woodside (7) have applied the excluded volume theory to ultracentrifuge-sedimentation theory for polyelectrolytes.

Synopsis

Previous equations developed for determining the concentration dependence and molecular weight of a polyelectrolyte from ultracentrifuge data were applied to a heterogeneous polymer system. The concentration coefficient of a polymer is $(B_r/2)(\bar{M}_w/\bar{M}_z)_r$ for $\bar{M}_{z,r}$ determinations. The B coefficients as obtained from $\bar{M}_n$, $\bar{M}_w$ and $\bar{M}_z$ have approximately the same relationships, Thus the slope of a plot of the reciprocal apparent molecular weight versus the polymer concentrations will depend not only on the type of molecular weight measured but also on the heterogeneity of the polymer. The concentration coefficient B includes terms for the interaction of polymer with polymer and polymer with low-molecular weight electrolyte. The main positive term for a polyelectrolyte, i.e., the $-(Z_P/M_D)$ $(d \ln C_B/dC_P)$ term, is not influenced by heterogeneity except in the (Z_P/M_D) expression, which is a weight-average value.

IV. THE DIPOLE MOMENT

Introduction

Equations were derived in Part I (13) which showed that in equilibrium ultracentrifugal molecular weight determinations the electrical potential term must be considered. By assuming that the activity coefficient is the product of three activity coefficients, i.e., $\bar{\gamma} = \gamma_N\gamma_d\gamma_E$, the electrical potential term becomes a function of the electrical charge (γ_E) and

the dipole moment (γ_d) of the polyelectrolyte. The third coefficient (γ_N) is due to nonelectrostatic interactions of the polymer. Because the polyelectrolyte contributes to ionic strength, the electrical potential will change with radius in an ultracentrifugal cell. This change in electrical potential or ionic strength will affect both coefficients γ_E and γ_d.

Let us consider a system containing a solvent plus a homogeneous polyelectrolyte PX_Z and a low molecular weight electrolyte BX where both PX_Z and BX completely dissociate. Then the equation for the ultracentrifuge molecular weight of the radius r can be expressed as (13):

$$\frac{1}{M_r^{app}} = \left(\frac{1}{M_{D,r}}\right) + B_{D,r}\, C_P \tag{1}$$

Here C_p is the polymer concentration, $\bar{M}_r^{app}$ and $\bar{M}_{D,r}$ are the apparent and extrapolated molecular weights and coefficient B is:

$$B_{D,r} = -\left(\frac{Z_P}{\bar{M}_D}\right)\left(\frac{d \ln C_B}{dC_P}\right) + \left(\frac{1}{RT}\right)\left(\frac{1}{\bar{M}_D dC_P}\right)(dW_N + dW_d + dW_E) \tag{2}$$

when the electrostatic charge Z_p does not vary with r. In equation 2 the electrostatic terms dW_d and dW_E and nonelectrostatic term dW_N can be expressed as (13, 15):

$$dW_j = \left[\left(\frac{\partial \ln \gamma_{j,PX_Z}}{\partial m_{PX_Z}}\right)_{T,p,m_{BX}} - Z_P\left(\frac{\partial \ln \gamma_{j,BX}}{\partial m_{PX_Z}}\right)_{T,p,m_{BX}}\right] dm_{PX_Z} + \left[\left(\frac{\partial \ln \gamma_{j,PX_Z}}{\partial m_{BX}}\right)_{T,p,m_{PX_Z}} - Z_P\left(\frac{\partial \ln \gamma_{j,BX}}{\partial m_{BX}}\right)_{T,p,m_{PX_Z}}\right] dm_{BX} \tag{3}$$

where the subscript j equals d, E or N, which in turn refer to the coefficients γ_d, γ_E or γN. The other subscripts refer to the polymer PX_Z or the salt BX. The letter m represents molality. Expressions for the electrical potential terms $\ln \gamma_{E,PX_Z}$ and $\ln \gamma_{d,PX_Z}$ will be developed from simple models.

Proposed Models

Because of the complications involved in the electrostatic term, various models must be proposed. Three models will be considered here. In order to simplify the calculations, it will be assumed in all cases that the valence Z_i of the point charges is equal to $Z_i = 1$.

Model 1: The electrostatic charges on the polymer are considered as being point charges, all being either positive or negative. Hence, the main effect is their contribution to the ionic strength. Model 2: The macroelectrolyte PX_Z is assumed to be a spherical ion having a radius $\bar{r}$ and a total charge Z_P. As in Model 1, no zwitterions are considered. Model 3: The polymer having a charge Z_P consists of small independent dipoles (or zwitterions) which are additive. As in the second model, it is assumed that the net electrostatic charge of the polymer contributes to the ionic strength as a single ion. For a polymer whose net charge is positive, the number of zwitterions or dipoles will be equal to the total positive charge minus the number of negative charges or λ_i. From equations 2 and 3 it is seen that the total electrical potential term W_i can be expressed as:

$$W_i = W_{E,i} + W_{d,i} = RT \ln \gamma_{E,i} + RT \ln \gamma_{d,i} \tag{4}$$

where $\gamma_{E,i}$ and $\gamma_{d,i}$ are coefficients caused by the electrostatic charge and the zwitterions, respectively. In the first and second models RT ln $\gamma_{d,i}$ may be zero and, hence, $W_i = RT \text{ in } \gamma_{E,i}$, while in the third model both $\gamma_{E,i}$ and $\gamma_{d,i}$ must be considered.

First Model. For the first model, let us assume that each point charge Z_i has a Poisson distribution of charges surrounding it and that the ionic strength is not greater than 0.01. Hence, the theory of Debye and Hückel can be applied directly (5, 36). For this case the value of W_i in equation 2 and subsequent equations is equal to $W_i = (1/2)(Z_i\epsilon)\psi$ where Ψ is the electrical potential per mole, Z_i is the valence and ϵ equals 4.803×10^{-10} e.s.u. If there are Z_p electrostatic charges on the polymer each having a valence of Z_p, then $W_i = (1/2) Z_p(Z_p\epsilon)\psi$. The electrical potential for each point charge at the ultracentrifuge radius r is than

$$(\psi/N) = \frac{Z_p\epsilon}{D\bar{r}_i} - \left(\frac{Z_p\epsilon}{D}\right)\left(\frac{K}{1+K\bar{r}_i}\right) \equiv -\left(\frac{Z_p\epsilon}{D}\right)\left(\frac{K}{1+K\bar{r}_i}\right) \qquad (5)$$

where N is Avogadro's number. Assuming that the electrostatic charges are remote from each other, then the value of $(Z_p\epsilon/D\bar{r}_i')$ is negligible (36). Hence, we shall be concerned only with that potential of the ion of charge $Z_p\epsilon$ which is due to the surrounding ions. The value of $\bar{r}_i$ is the distance in centimeter units from the center of one ion to the center of another ion at its surface. D is the dielectric constant of the surrounding medium and for simplicity can be assumed to be equal to that of the solvent molecules, i.e., $D = D_o = 78.54$ for water at 25°. The value of K can then be obtained from (12, 35)

$$K = \left[\frac{8\pi N^2\epsilon^2 S}{(1000)DRT}\right]^{1/2} = \left(0.3288 \times 10^{8}\right)(S^{1/2}) \qquad (6)$$

for an aqueous solution at 25°. Here S = the ionic strength $= (1/2)\Sigma n_i Z_i^2$ where n_i is in moles per liter. For the polymer PX_Z the value of n_p, assuming point charges, equals $(1000\ Z_P\ C_P/MPX_Z)$ where C_P is in g/ml. In terms of the electrolyte instead of the constituent ions, the value of W_i as obtained from equations 4 and 5 becomes for PX_Z:

$$W_{\epsilon,PX_Z} = RT \ln \gamma_{\epsilon,PX_Z} = \frac{Z_p\epsilon\psi}{2} = \left(\frac{Z_p Z_p Z_X \epsilon^2}{2D}\right)\left(\frac{NK}{1+K\bar{r}_{PX_Z}}\right) \qquad (7)$$

where (Z_pZ_X) is always negative. An equivalent expression occurs for BX.

If it is assumed that the electrostatic charge Z_p of the polymer does not vary appreciably with cell radius, that the valeuces Z_p, Z_X, and Z_B are unity and, in addition, that the value of $K\bar{r}$ is much less than one, then the value of W_{E,PX_Z} for the polymer is:

$$W_{\epsilon,PX_Z} = RT \ln \gamma_{\epsilon,PX_Z} = -Z_p k_\epsilon (S^{1/2}/D) \qquad (8.0)$$

where

$$S = (0.5 \times 10^{3})\left[Z_P^2\left(Z_P C_P/M_{PX_Z}\right) + Z_X^2\left(Z_P C_P/M_{PX_Z}\right) + Z_B^2\left(C_B/M_{BX}\right) + Z_X^2\left(C_B/M_{BX}\right)\right]$$

$$= 10^{3}\left[\left(Z_P C_P/M_{PX_Z}\right) + \left(C_B/M_{BX}\right)\right] \qquad (8.1)$$

and where

$$k_\epsilon = \left(0.1644 \times 10^{8}\right)(N\epsilon^2) \qquad (8.2)$$

as obtained from equations 6 and 7. For the salt BX the value of $W_{E,BX}$ becomes

$$W_{E,BX} = RT \ln \gamma_{E,BX} = -k_E (S^{1/2}/D) \tag{9}$$

where $(S^{\frac{1}{2}})$ and k_E are defined as given in equations 8.1 and 8.2. Substitution of these values of $W_{E,i}$ into equation 3 gives:

$$dW_E = (k_E)\left[Z_P \left(\frac{\partial (S^{1/2}/D)}{\partial C_{PX_Z}}\right)_{T,P,C_{BX}} - Z_P \left(\frac{\partial (S^{1/2}/D)}{\partial C_{PX_Z}}\right)_{T,P,C_{BX}}\right] dC_{PX_Z}$$
$$+ (k_E)\left[Z_P \left(\frac{\partial (S^{1/2}/D)}{\partial C_{BX}}\right)_{T,P,C_{PX_Z}} - Z_P \left(\frac{\partial (S^{1/2}/D)}{\partial C_{BX}}\right)_{T,P,C_{PX_Z}}\right] dC_{BX} = 0 \tag{10}$$

Thus for the first model where the polymer behaves as point charges and where dipole-dipole interactions are absent, the resulting change in the electrical potential W_E is zero.

Second Model. In the second model, the large radius of the polymer may make the value of $K\bar{r}_{PX_Z}$ in equation 7 significant and, hence, $K\bar{r}_{PX_Z}$ cannot be neglected. However, if the radius r_B for the ion B^+ (or X^-) is defined as the distance from the center of the ion B^+ (or X^-) to the center of the ion P^{+Z}, then the radius r_P equals the radius r_B. Using this definition we again obtain the same expression for W_{E,PX_Z} and $Z_P W_{E,BX}$. Hence, dW_E again equals zero.

If $r_P >> r_B$, then the term $(d W_E/RTM_D dC_P)$ of equation 2 is unequal to zero. In order to approximate the magnitude of this $d W_E$ term, let us assume that the cross-coefficients of equation 3 are negligible. Equation 3 then becomes:

$$dW_E = d \ln \gamma_{E,PX_Z} - Z_P d \ln \gamma_{E,BX}$$

Using equation 7 and assuming that $d W_E = (W_E)_b - (W_E)_a$ where "a" represents the meniscus and "b" the cell bottom, then the $d W_E$ term of equation 2 becomes for an aqueous solution at 25°:

$$\left(\frac{dW_E}{RT M_D dC_P}\right) = -(1.174)\left(\frac{Z_P}{M_D \Delta C_P}\right)\left\{\Delta\left(\frac{S^{1/2}}{1+0.3288\, S^{1/2} r^*_{PX_Z}}\right) - \Delta\left(\frac{S^{1/2}}{1+0.3288\, S^{1/2} r^*_{BX}}\right)\right\} \tag{11}$$

where $r^*_{PX_Z}$ and r^*_{BX} are in Ångstrom units. The delta in equation 11 refers to the value at the radius r = b minus that at r = a. In equation 11 the ionic strength S will be different than the ionic strength as expressed in equation 8.1 since now the valence of the polymer P^{+Z} is Z_P instead of $Z_P = 1$. Thus for equation 11 the value of S is:

$$S = (0.5 \times 10^3)\left[Z_P (1+Z_P)(C_P/M_{PX_Z}) + 2 (C_B/M_{BX})\right] \tag{12}$$

where C_P and C_B are in g/ml.

Let us consider bovine serum albumin at pH 3.8 in an aqueous HCl solution in the absence of added salt (12). Here $Z_P = 20$, λ = number of dipoles = 80, $M_D \equiv M_P = 69,000$ and $(C_{P,b} - C_{P,a}) = \Delta C_P = 0.32 \times 10^{-2}$ g/ml when $C_{P,a} = 0.11 \times 10^{-2}$ g/ml and $C_{P,b} = 0.43 \times 10^{-2}$ g/ml. According to equation 12 the ionic strength at the meniscus is $S_a = 3.5 \times 10^{-3}$ and at the cell bottom is $S_b = 13.2 \times 10^{-3}$. Assuming that $r^*_{PX_Z}$ is 70Å (35) and that $K\bar{r}_{BX}$ is negligible in comparison to one, then the value of $(d W_E/RTM_D\, d C_P)$ according to equation 11 is 2.6×10^{-3}. The experimental B coefficient for bovine serum albumin under the conditions used was $B = 3.7 \times 10^{-3}$ when C_P is in units of g/ml (12). Hence, the value above of 2.6×10^{-3} for the $d W_E$ term is comparable in magnitude.

The $-(Z_P/M_D)(d \ln C_B/dC_P)$ term in equation 2 can be approximated as:

$$-\left(\frac{Z_P}{M_D}\right)\left(\frac{d \ln C_B}{dC_P}\right) = \left(\frac{1}{2}\right)\left(\frac{Z_P}{M_P}\right)^2\left(\frac{M_B}{C_B}\right) \tag{13}$$

The value of this term for the example where (C_B/M_B) equals 1.6×10^{-7} moles/ml is $-(Z_P/M_D)(d \ln C_B/d C_P) = 260 \times 10^{-3}$ using equation 13. This value is approximately 70 times larger than the experimental B coefficient and 100 times larger than the $d W_E$ term. In addition, the $d W_E$ term is positive and, hence, cannot account for the low experimental value.

Third Model. In this model the polyelectrolyte possesses dipolar ions, but the low molecular weight electrolyte does not. Since the activity of the i^{th} species is now a function of dipolar ions as well as ions, then the activity coefficient $\gamma_{d,i}$ must be considered in equation 2. In treating the dipolar ion, we can assume Kirkwood's (30) spherical model having a radius $\bar{b}$ and a point dipole of moment μ at its center (see discussion by Kirkwood (30) and Edsall and Wyman (6), p. 304). The distance of closest approach of the dipolar ion and a surrounding ion is given as $\bar{a}$. Then according to Kirkwood's (30) equation 12 (or equation 88 of Edsall and Wyman (5)) the limiting law valid for infinite dilution for the activity coefficient of a dipolar ion is

$$W_{d,i} = RT \ln \gamma_{d,i} = -(S)\left[\frac{2\pi N^2 \epsilon^2}{1000\, D}\right]\left[\left(\frac{3}{2}\right)\left(\frac{\mu^2}{D\bar{a}kT}\right) - \left(\frac{\bar{b}^3 \alpha(\rho)}{\bar{a}}\right)\right] \tag{14}$$

where $\rho = (\bar{b}/\bar{a})$ and $\alpha(\rho)$ is a function of ρ. The activity coefficient of a salt or the counterion due to the presence of the zwitterions is very small in comparison to the activity coefficient of the zwitterion. (Compare the magnitude of the second term of Kirkwood's (29) equation 21 with his equation 24.) Hence, the two $\gamma_{d,BX}$ terms of equation 3 can be neglected. In addition, it can be assumed that the cross coefficient term $(\partial \ln \gamma_{d,PX_Z}/\partial m_{BX})dm_{BX}$ is negligible. The only remaining term is $(\partial \ln \gamma_{d,PX_Z}/\partial m_{PX_Z})dm_{PX_Z}$ Thus from the definition of $d W_d$ as given in equations 2 and 3, the value of $d W_d$ is $d W_d = RT\, d \ln \gamma_{d,pX_Z}$.

If the λ_i dipoles for each i^{th} species are additive, then $\lambda_i RT \ln \gamma_{d,i}$ represents the total dipolar ion effect. Thus the value of $(d W_d/RT M_D d C_p)_r$ as obtained from equations 2, 3 and 14 is:

$$\left(\frac{dW_d}{RT M_D dC_P}\right) = -\left[\frac{d(\lambda_i S/D)}{RTM_D dC_P}\right]\left[\frac{2\pi N^2 \epsilon^2}{1000}\right]\left[\left(\frac{3}{2}\right)\left(\frac{\mu^2}{D\bar{a}kT}\right) - \left(\frac{\bar{b}^3 \alpha(\rho)}{\bar{a}}\right)\right] \tag{15}$$

where $k = 1.380 \times 10^{-16}$ and the ionic strength S is defined as given in equation 12. If λ_i and the dielectric constant D do not vary with the radius, then equation 15 can be put into the form:

$$\left(\frac{\Delta W_D}{RTM_D \Delta C_P}\right) = -(0.02702)\left[\frac{\lambda_i \Delta S}{M_D \Delta C_P}\right]\left[\frac{(0.4644)(\mu^{*2}) - (\bar{b}^*)^3\, \alpha(\rho)}{\bar{a}^*}\right] \tag{16}$$

assuming that $d S = S_b - S_a = \Delta S$, that $d C_p = C_{p,b} - C_{p,a} = \Delta C_p$ and that $d W_d = (W_d)_b - (W_d)_b = \Delta W_D$. Here μ^* is in Debye units, a^* and b^* are in Ångstrom units and ΔC_p is in g/ml.

The value of the (dW_d/dC_p) term as given in equations 15 and 16 should remain essentially constant with a change in the polymer concentration.

That is, when the change in ionic strength is primarily due to a change in polymer concentration, then the quantity (dS/dC_p) in equation 15 will become:

$$\frac{dS}{dC_p} = \frac{d\left[Z_p^2(C_p/M_{PX_Z}) + Z_X^2\,(Z_pC_p/M_{PX_Z})\right](10^3)}{dC_p} = Z_p(1+Z_p)(10^3)/M_{PX_Z} \qquad (17)$$

The term (dS/dC_p) should be constant even at zero polymer concentration. For example, the positive part of the B coefficient, i.e., the term $-(Z_p/M_D)\, d \ln C_B/dC_p$ as given in equation 2, can be approximated as $(Z_p/M_D)(1/C_B)(C_{B,a} - C_{B,b})/(C_{p,b} - C_{p,a})$. This approximation suggests that since the B coefficient is constant, then the ratio of the difference between the concentration of the buffer at the meniscus and that at the cell bottom divided by the corresponding difference of the polymer concentrations should remain constant as the polymer concentration approaches zero. Hence, if the polymer quantity $Z_p^2(C_{p,b} - C_{p,a})$ in the ionic strength term ΔS is greater than the buffer quantity $Z_B^2(C_{B,a} - C_{B,b})$ at finite polymer concentrations, then it should still be greater at infinitely dilute polymer concentrations. Thus in most cases the term $(d S/d C_p)$ will be equal to $[Z_p(1 + Z_p)(1000)/M_{PX_Z})]$ at all polymer concentrations.

In order to examine the magnitude of the electrostatic term given in equation 38, let us consider the example given for bovine serum albumin at pH 3.8 in an aqueous HCl solution (12). Assuming that $b \equiv 6$Å and that $\rho = (\bar{b}/\bar{a}) = 0.9$, then $\bar{a} \equiv 6.7$ Å and $\alpha(\rho) = 1.96$ (30). In addition, it may be assumed that $\mu^* = 40$ Debye units (see reference 5, p.672). If the polymer has a sufficiently large charge, then the change in ionic strength from the meniscus to the cell bottom at finite polymer concentrations will be due mainly to the polymer and not to the low molecular weight electrolyte. Hence, it may be assumed that the change in the concentration of BX is negligible. Thus $(d S/dC_p)$ can be obtained from equation 17. Substitution of the above values into equation 16 gives: $[\Delta W_D/RT\, M_D \Delta C_p] = -4.5 \times 10^{-3}$. The observed B coefficient for bovine serum albumin was $B = 3.7 \times 10^{-3}$ when C_p is g/ml (30). This electrostatic effect of -4.5×10^{-3} would therefore reduce the observed B coefficient by more than one-half. If the dipole moment is $\mu^* = 100$ Debye units, then $[\Delta W_D/RT\, M_D \Delta C_p] = -61 \times 10^{-3}$. In this case the B coefficient has been lowered to one-sixteenth of its original value by the electrostatic potential.

These calculations assume that each small dipole is additive. However, the polyelectrolyte could—and most likely does—behave as a single, large dipole. This behavior would give an enormous effect and could readily account for the large discrepancy between the theoretical B coefficient as calculated from equation 13 and the experimental B coefficient.

The third model, which incorporates the dipole moment, may be elaborated to determine the effect of a large dipole by using Kirkwood's (29) equation 21 for $RT \ln \gamma$. Kirkwood's model is a spherical polyelectrolyte containing many positive and negative charges that have a specific distribution. The first term in his (29) equation 21 is due to the net electrostatic charge Z_p and corresponds to the $d W_{E,PX_Z}$ term while the second term is due to the dipolar ion effect, i.e., $d W_{d,PX_Z}$. Examination of this model was not made because the exact spatial distribution of charges for bovine serum albumin is not known.

Discussion

The first model for a charged polymer suggests that the electrostatic term of d W_E of equation 2 is zero. Calculations for the second model, which considers the difference in radii of the polyelectrolyte and the salt, indicate that the dW_E term is positive and very small in comparison to the theoretical B coefficient. Hence, the third model, which incorporates a negative dipole moment term dW_d, is the only model that can explain the large discrepancy between the experimental and theoretical B coefficients. Thus it is not necessary to use an "effective" charge to explain the low experimental B coefficients of some polyelectrolytes. (See discussion in Part I). Those polyelectrolytes that do not have a dipole moment should give a B coefficient that can be correlated with the $-(Z_p/M_D)(d \ln C_B/d C_p)$ term of equation 2. Calculations (14) given previously based on the experimental work of Johnson et al. (26), suggest that silicotungstic acid may be a polyelectrolyte which exhibits no dipole moment and electrostatic effects, i.e., $d W_E \cong d W_d \cong 0$. With this polyelectrolyte the effective charge is equal to the charge obtained by titration. This experimental result suggests that the counterion does not reduce the charge of an electrostatically charged polymer. In contrast with these results on silicotungstic acid, such proteins as bovine serum albumin (12) and Proteus vulgaris flagellin (11) exhibit large dipolar ion effects, which one would expect on the basis of the d W_d term. The experimentally low concentration coefficients observed on some charged polymers can therefore be explained on the basis of a dipolar ion effect. This effect is due to a change in the ionic strength of the medium with a change in the ultracentrifugal radius (see equation 15).

When $Z_p = 0$ but zwitterions exist on the polymer, then the concentration dependence due to the dW_d term will depend on the change in the concentration of the low molecular weight electrolyte with the cell radius. Therefore a zero net charge does not necessarily exclude electrostatic effects for a polymer. It was concluded in the discussion of the third model that the change in ionic strength divided by a change in polymer concentration in the dW_d term should remain constant as the polymer concentration approaches zero. Since in equation 1 the dW_d term is multiplied by C_p, then (d W_d) should approach zero as C_p approaches zero. This deduction is based on the fact that the Donnan equilibrium effect of the polymer on the salt maintains a constant ratio of (d C_B/ d C_p). Hence, for a charged polymer the dipolar effect should not influence the extrapolated molecular weight. This effect may not be true for an uncharged polymer that contains zwitterions since now the Donnan electrostatic effect between the polymer and salt is absent. Therefore, for $Z_p = 0$ and $d W_d \neq 0$ there may exist a dipolar ion effect independent of the polymer concentration. This electrostatic effect would be caused by the change in the concentration of BX with cell radius. The extrapolated molecular weight $M_{D,r}$ will then be

$$M_{D,r} = M_{PX_Z}\left[1-\left(\frac{Z_p-\Gamma}{M_{PX_Z}}\right)M_{BX}Q - \left(\frac{2}{\omega^2(1-\bar{V}_{PX_Z}\rho)M_{PX_Z}}\right)\left(\frac{dW_d}{d(r^2)}\right)\right] \qquad (18)$$

Here d W_d is defined as given in equation 15, Q is $(1-\bar{V}_{BX}\rho)/(1-\bar{V}_{PX_Z}\rho)$

Γ is the binding coefficient (17) and M_{BX} is the molecular weight of the low molecular weight electrolyte.

Let us now examine how the dipolar ion effect will influence the concentration dependence. As noted, the d W_d term is negative and, hence, will reduce the B coefficient. If it is assumed that the polymer consists of small spherical dipoles, then the definition of d W_d is given in equations 15 and 16. The term $(3/2)(\mu^2/D\bar{a}kT)$ can be considered as the salting-in term and the quantity $-(\bar{b}^3\alpha(\rho)/\bar{a})$ as the salting-out term (5). As discussed, the value (d S/d C_p) is proportional to $Z_p(1 + Z_p)$, and hence, this term will not be influenced significantly by an increase in the concentration of BX. The main effect on the value of d W_d as BX is increased will be on the salting-in and salting-out terms. Hence, the value of d W_d will approach zero as the magnitude of the salting-out term approaches that of the salting-in term. Because the quantity d W_d is negative, a decrease in its value will increase the value of the B coefficient. However, when the concentration of BX is increased, both the negative d W_d term and the opposing positive (-d ln C_B/d C_p) term will approach zero. As the concentration of BX is increased the value of the B coefficient should decrease.

The magnitude of the salting-out term must increase for different salts according to the Hofmeister series $CaCl_2 < NaCl < (NH_4)_2SO_4 < Na_2SO_4$. (5) Thus the B coefficient may be smaller in the presence of $CaCl_2$ than in the presence of NaCl. That is, the magnitude of d W_d will increase in going from a NaCl to a $CaCl_2$ solution which in turn will decrease the coefficient B. However, the effect of a change in the properties of BX on the (-d ln C_B/d C_p) term must also be considered.

Synopsis

It was previously shown that in ultracentrifugal molecular weight determinations there exists an electrical potential term in the concentration coefficient. This electrical potential term is a function of both the electrostatic charge (dW_E) and the dipole moment (dW_d) of a polymer. While the magnitude of the dW_E term appears small, that of the dW_d term could be extremely large for polymers containing a large number of positive and negative charges (zwitterions). A constant factor that effects the extrapolated molecular weight may exist in the case of a zwitterion where the net charge is zero.

V. THE BINDING COEFFICIENT

Introduction

The preferential binding of one type of solvent molecule by a polymer in a binary solvent system has been treated by Wales and Williams (53) and Williams et al. (55), for equilibrium ultracentrifugation. These equations can also be applied directly to a polyelectrolyte PX_z in a binary solvent system. In addition to this problem of solvation, a polyelectrolyte may bind ions to its surface. The effect of such binding may be similar to the binding of an un-ionized solvent or solute particle. How-

ever, different conditions arise in the case of the binding of ions because such ions can act as common ions or counterions to the polyelectrolyte. The general equations derived in Part I (13) will now be applied to the binding of a salt BX to a polymer PX_Z where both polymer and salt completely dissociate in the solvent to produce the common counterion X.

The Binding Coefficient

Let us consider the case where the activity coefficients of B^+ and X^- are constant and where the binding occurs only between the homogeneous polymer PX_Z and the low molecular weight electrolyte BX, binding between the same species being negligible. By using equations 3.5 and 3.6 of reference 13, the expressions for the homogeneous polymer and BX become:

$$2A_{PX_Z}rdr = d\ln m_P\gamma_{N,P} + d(Z_P \ln m_X) + \left(\frac{1}{RT}\right)\left(dW_{PX_Z}\right) + \left(\frac{1}{RT}\right)\left(\frac{\partial\mu_{PX_Z}}{\partial m_{BX}}\right)_{T,p,m_k} dm_{BX} \tag{1}$$

$$2A_{BX}rdr = \left(\frac{1}{RT}\right)\left(\frac{\partial\mu_{BX}}{\partial m_{BX}}\right)_{T,p,m_k} dm_{BX} + \left(\frac{1}{RT}\right)\left(\frac{\partial\mu_{BX}}{\partial m_{PX_Z}}\right)_{T,p,m_k} dm_{PX_Z} + \left(\frac{1}{RT}\right)\left(dW_{BX}\right) \tag{2}$$

According to Scatchard (44) the term $(\partial\mu_{PX_Z}/\partial m_{BX})_{T,p,m_k}$ is equivalent to the term $(\partial\mu_{BX}/\partial m_{PX_Z})_{T,p,m_k}$. By using the method given by Williams et al. (55), the term $(\partial\mu_{PX_Z}/\partial m_{BX})$ in equation 1 can be eliminated by making the following substitution into equations 1 and 2:

$$\left(\frac{\partial\mu_{PX_Z}}{\partial m_{BX}}\right)_{T,p,m_k} = \left(\frac{\partial\mu_{BX}}{\partial m_{PX_Z}}\right)_{T,p,m_k} = \frac{\left(\frac{\partial\mu_{BX}}{\partial m_{PX_Z}}\right)_{T,p,m_k}}{\left(\frac{\partial\mu_{BX}}{\partial m_{BX}}\right)_{T,p,m_k}}\left[\frac{\partial\mu_{BX}}{\partial m_{BX}}\right]_{T,p,m_k} = -\Gamma\left[\frac{\partial\mu_{BX}}{\partial m_{BX}}\right]_{T,p,m_k} \tag{3}$$

Here Γ is the binding coefficient (55) and is equal to:

$$\Gamma = \left(\partial m_{BX}/\partial m_{PX_Z}\right)_{T,p,\mu_{PX_Z}} \tag{4}$$

Substitution of equation 3 into equations 1 and 2, multiplication of resulting equation 2 by Γ and adding the two modified equations 1 and 2 gives:

$$\left(A_{PX_Z} + \Gamma A_{BX}\right)\left(2\,rdr\right) = \left[1-\Gamma^2\left(\frac{m_{PX_Z}}{m_{BX}}\right)\right]\left[d\ln m_P + \ln m_X\, dZ_P + Z_P\, d\ln m_X\right] + d\ln\gamma_{N,P} + \left(\frac{1}{RT}\right)\left[dW_{PX_Z} + \Gamma dW_{BX}\right] \tag{5}$$

since $(\partial\mu_{BX}/\partial m_{BX})_{T,p,m_k}$ equals $(1/m_{N,BX})$ if γ_{BX} is constant. The term d ln m_X in equation 5 can be obtained from equation 2:

$$d\ln m_X = \frac{\left(2A_{BX}rdr\right) - d\ln m_B - \left(1/RT\right)dW_{BX} + \Gamma\left(m_{PX_Z}/m_{BX}\right)\left(d\ln m_P + \ln m_X dZ_P\right)}{\left[1-\left(Z_P\right)\left(\Gamma\right)\left(m_{PX_Z}/m_{BX}\right)\right]} \tag{6}$$

Substitution of the value of d ln m_X given in equation 6 into equation 5 yields:

$$\left(A_{PX_Z}+\Gamma A_{BX}\right)\left(2\,rdr\right) = -F\left(d\ln m_B\right) + d\ln\gamma_{N,P} + \left(\frac{1}{RT}\right)\left[dW_{PX_Z} - \left(FZ_P - \Gamma\right)dW_{BX}\right]$$
$$+ F\left(d\ln m_P + \ln m_X\, dZ_P\right) + FZ_P\left(2A_{BX}rdr\right) \tag{7}$$

where

$$F=\left[1-\Gamma^2\left(m_{PX_Z}/m_{BX}\right)\right]\Big/\left[1-Z_P\Gamma\left(m_{PX_Z}/m_{BX}\right)\right]=1+(Z-\Gamma)\,HC_P \tag{8}$$

and where

$$H=(1/C_P)\,\Gamma\left(m_{PX_Z}/m_{BX}\right)\Big/\left[1-Z_P\Gamma\left(m_{PX_Z}/m_{BX}\right)\right]$$

or

$$H=\Gamma\left(M_{BX}/C_BM_{PX_Z}\right)\Big/\left[1-Z_P\Gamma\left(C_PM_{BX}/C_BM_{PX_Z}\right)\right] \tag{9}$$

if molalities are equal to molarities. If we substitute this value of F into equation 7 and assume that molalities equal molarities, then equation 7 becomes:

$$\begin{aligned}\left\{2\left[\bar{A}_{PX_Z}-(Z_P-\Gamma)\bar{A}_{BX}\right]\right\}=&\left(\frac{1}{rdr}\right)\left(\frac{dC_P}{C_P}\right)\Big\{1+(Z_P-\Gamma)\,HC_P+\left(\frac{d\ln\gamma_{M,P}}{dC_P}\right)C_P+\left[1+(Z_P-\Gamma)\,HC_P\right]\\&\left(\ln\left[\frac{C_B}{M_{BX}}\right)+\left(\frac{Z_P}{M_{PX_Z}}\right)C_P\right]\Big)\left(\frac{dZ_P}{dC_P}\right)C_P+(Z_P-\Gamma)HZ_P\left(\frac{M_{BX}}{M_{PX_Z}}\right)QC_P^2+\left(\frac{1}{RT}\right)\left[\left(\frac{dW_{PX_Z}}{dC_P}\right)-(Z_P-\Gamma)(1+Z_PHC_P)\right.\\&\left.\left(\frac{dW_{BX}}{dC_P}\right)\right]C_P-\left[1+(Z_P-\Gamma)\,HC_P\right]Z_P\left(\frac{d\ln C_B}{dC_P}\right)C_P\Big\}\end{aligned} \tag{10}$$

In equation 10 the term $\left[\bar{A}_{BX}(2\,rdr)/dC_P\right]$ was converted to $(M_{BX}/M_{PX_Z})(Q/C_P)$ where $Q=(1-\bar{V}_{BX}\rho)/(1-\bar{V}_{PX_Z}\rho)$ by assuming that the concentration coefficient is negligible in this B coefficient term.

In terms of molecular weights, equation 10 becomes:

$$\frac{1}{M_{P,r}^{app}}=\frac{1}{M_{\Gamma,r}}+B_{\Gamma,r}\,C_{P,r} \tag{11}$$

where

$$\begin{aligned}B_{\Gamma,r}=&\left[1+(Z_P-\Gamma)\,HC_P\right]_r\Big\{\ln\left[\frac{C_B}{M_{BX}}\right)+\left(\frac{Z_PC_P}{M_{PX_Z}}\right)\Big]_r\left[\frac{d\left(\frac{Z_P}{M_\Gamma}\right)}{dC_P}\right]_r-\left[1+(Z_P-\Gamma)HC_P\right]\left(\frac{Z_P}{M_\Gamma}\right)\left(\frac{d\ln C_B}{dC_P}\right)_r+\left(\frac{1}{RT}\right)\left(\frac{dW_\Gamma}{M_\Gamma\,dC_P}\right)_r\\&+\left(\frac{d\ln\gamma_{M,P}}{M_\Gamma\,dC_P}\right)_r+\left[\frac{(Z_P-\Gamma)H}{M_\Gamma}\right]_r\left[1+\left(\frac{Z_P}{M_{PX_Z}}\right)QM_{BX}C_P\right]\end{aligned} \tag{12}$$

and

$$dW_\Gamma=dW_{PX_Z}-(Z_P-\Gamma)(1+Z_PHC_P)\,dW_{BX} \tag{13}$$

and

$$M_{P,r}^{app}=\left(\frac{1}{C_{P,r}}\right)\left(\frac{dC_{P,r}}{rdr}\right)\left[\frac{RT}{(1-\bar{V}_{PX_Z}\rho)\,\omega^2}\right] \tag{14}$$

and

$$M_{\Gamma,r}=M_{PX_Z}\left[1-(Z_P-\Gamma)\left(\frac{M_{BX}}{M_{PX_Z}}\right)\left(\frac{1-\bar{V}_{BX}\rho}{1-\bar{V}_{PX_Z}\rho}\right)\right]_r \tag{15}$$

The terms Γ and H are defined as given in equations 4 and 9. If the electrostatic charge Z_P is equal to the number of molecules of BX bound to the polymer P^{+Z}, then equation 11 becomes:

$$\frac{1}{M^{app}_{p,r}} = \frac{1}{M_{PX_Z}} + B'_{D,r}\, C_{p,r} \quad (\text{for } \Gamma = Z_p) \tag{16}$$

where $M^{app}_{p,r}$ is defined in equation 14. Thus, the extrapolated molecular weight equals the polymer molecular weight when $\Gamma = Z_p$. The coefficient $B'_{p,r}$ is the same as the $B_{D,r}$ coefficient of equation 7.1 of Part I except for the electrostatic term dW_D. In equation 7.1 of Part I, the value of dW_D is $(dW_{PX_Z} - Z_p dW_{BX})$ while in equation 16 where $\Gamma = Z_p$ the value of dW_Γ is dW_{PX_Z}. The discrepancy is most likely negligible (see calculation given in Part IV). Hence, the $B'_{D,r}$ coefficient of equation 16 is essentially the same as the $B_{D,r}$ coefficient of equation 7.1 of Part I.

The equations given above can be elaborated if the activity coefficients of the low molecular weight electrolyte (55) are considered or if a heterogeneous polymer (53) is considered.

Four Component Systems

Consider the addition of a low molecular weight electrolyte GX to a system containing the polymer PX_Z plus a low molecular weight electrolyte BX. Equation 2 could be expressed first in terms of BX and then in terms of GX. If these two equations are added one obtains:

$$d\ln (m_B m_G)^{1/2} + d\ln m_X + \left(\frac{1}{2RT}\right)(dW_{BX} + dW_{GX}) = (A_{BX} + A_{GX})\, r\, dr \tag{17}$$

Equation 17 can be multiplied by Z_p and subtracted from equation 1. The result is that the term $M_{BX}(1-\bar{V}_{BX}\rho)$ in equation 15 for the definition of $M_{\Gamma,r}$ is replaced by $\left[M_{BX}(1-\bar{V}_{BX}\rho) + M_{GX}(1-\bar{V}_{GX}\rho)\right](1/2)$ and the terms (C_B/M_B) and C_B in equation 12 for the definition of $B_{\Gamma,r}$ are replaced by $(C_B C_G/M_M M_G)^{1/2}$ and $(C_B C_G)^{1/2}$, respectively. Hence, if more than one salt is present, the averages given can be used. This type of averaging would also apply to the development of B_Γ for heterogeneous polymer systems (6) and also to equation 11 when both or only one low molecular electrolyte "bind" to the polymer. According to Scatchard (44) the following relationship for such a complex system should hold:

$$\left(\frac{\partial \mu_i}{\partial m_j}\right)_{T,p,m_k} = \left(\frac{\partial \mu_j}{\partial m_i}\right)_{T,p,m_k} \tag{18}$$

Hence, the binding coefficient terms of equation 1 become:

$$\left(\frac{1}{RT}\right)\left(\frac{\partial \mu_{PX_Z}}{\partial m_{BX}}\right)_{T,p,m_k} dm_{BX} + \left(\frac{1}{RT}\right)\left(\frac{\partial \mu_{PX_Z}}{\partial m_{GX}}\right)_{T,p,m_k} dm_{GX} = -\left[\left(\frac{\Gamma_{BX}}{m_{BX}}\right) dm_{BX} + \left(\frac{\Gamma_{GX}}{m_{GX}}\right) dm_{GX}\right] \tag{19}$$

For equation 17 when the binding coefficient is not zero and when γ_{BX} and γ_{GX} are constant, these terms become:

$$\left(\frac{1}{2RT}\right)\left[\left(\frac{\partial \mu_{BX}}{\partial m_{PX_Z}}\right)_{T,p,m_k} + \left(\frac{\partial \mu_{GX}}{\partial m_{PX_Z}}\right)_{T,p,m_k}\right] dm_{PX_Z} = -\left(\frac{1}{2}\right)\left[\left(\frac{\Gamma_{BX}}{m_{BX}}\right) + \left(\frac{\Gamma_{GX}}{m_{GX}}\right)\right] dm_{PX_Z} \tag{20}$$

where $\Gamma_{BX} = (\partial m_{BX}/\partial m_{PX_Z})_{T,p,\mu_{PX_Z}}$

and $\Gamma_{GX} = (\partial m_{GX}/\partial m_{PX_Z})_{T,p,\mu_{PX_Z}}$

Thus the term $\left(\frac{1}{2}\right)\left[\left(\frac{\Gamma_{BX}}{m_{BX}}\right)+\left(\frac{\Gamma_{GX}}{m_{GX}}\right)\right]=\left(\frac{1}{2}\right)\left[\Gamma_{BX}m_{GX}+\Gamma_{GX}m_{BX}\right]\Big/\left[m_{GX}+m_{BX}\right]$ would replace the term Γ_{BX}/m_{BX} in equation 5 and subsequent equations. If only one component binds to the polymer PX_Z, then the concentrations and molecular weights of both salts are averaged as given in equation 17 and the binding coefficient term simplifies to that for a single low molecular weight species.

Discussion

The extrapolated molecular weight and the concentration coefficient of a polymer PX_Z having a net charge Z_p are given in equations 11, 12 and 15. For the special case where the number of bound molecules of BX is equal to the net charge Z_p, the extrapolated molecular weight equals the molecular weight of the polymer (M_{PX_Z}) and the concentration coefficient equals that of a polymer PX_Z that has not bound any salt. Thus when $\Gamma = Z_p$, the only difference in experimental results for a polymer that binds in one system and does not bind in another similar system is in the extrapolated molecular weight.

Two types of "binding" between polymer and salt may occur: first, strong binding such as chelation of an ion with a specific site and, secondly, a loose association of the salt around the polymer. In the first case, the strong binding between the polymer and salt will most likely alter the net electrostatic charge Z_p. In such a case, Z_p may be reduced to zero, and hence, the extrapolated molecular weight will become greater than the polymer molecular weight. The second case—where a loose association occurs—is more difficult to explain with respect to how such an association can exist and how it affects the net charge of the polymer. As pointed out by Wallis and Record (57) the salt may be associated with the polyelectrolyte by a Donnan equilibrium effect. Recent work by Lapanje et al. (34)*, suggests that the low activity coefficient of the counterion is due to a close association of the counterion with the charges in the polyelectrolyte. However, discrete ion-pair formation between counterion and polymer appears to be ruled out (34). Thus, if ions from the salt BX are present in the polymer system, then these ions could readily exchange with the counterion. In order to maintain electroneutrality both B^+ and X^- would have to approach the charge on the polymer. A positive electrostatic charge on a polymer could therefore be associated with its counterion X^- followed by the near proximity of the B^+ ion and then the X^- ion. Vrij and Overbeek (52) concluded that the difference between the amounts of counterions and co-ions just compensates the particle charge Z_p. As in the case of binary solvent systems where one

*Their results however may be open to question since in their expression for the activity coefficient of the polyion or the bolion (equations 34 and 35 of reference 60) they neglect the possibility of one polymer inducing a dipole moment on another polymer as the two approach. Their low experimental counterion activity may therefore be induced polarization of the polyion, i.e., a change in the activity of the polyion and not a change in the activity coefficient of the counterion.

solvent may associate more strongly with the uncharged polymer, this loose association between polyelectrolyte and salt may also give the effect of binding. For a polyelectrolyte the apparent binding coefficient may then be equal to the net charge: $\Gamma_{BX} = Z_P$.

For a system containing silicotungstic acid plus a supporting electrolyte the electrostatic charge of the silicotungstic acid equals that obtained by titration studies (26). Since the charge was obtained from the concentration coefficient (26, 14), then binding may be present or absent, i.e., $\Gamma = 0$ or $\Gamma = Z_P$. By obtaining the extrapolated molecular weight on such polymers as silicotungstic acid, it may be possible to verify whether the surrounding ions of a polymer P^{+z} contribute to the binding coefficient.

Synopsis

Equilibrium ultracentrifugal equations for a solvent system containing a polyelectrolyte PX_z and a low molecular weight electrolyte BX were examined for the case where BX may bind or complex to the charged polymer. Alterations occurring when PX_z is present in a system containing two salts, BX or GX, where one or both may complex with PX_z were also considered. When the binding coefficient equals the net charge of the polymer P^{+z}, then the concentration coefficient equals that obtained for the same polymer system where binding is absent. Also the extrapolated molecular weight equals the true molecular weight. The equations developed may apply to all systems containing a polyelectrolyte and a salt.

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SCOTCH PINE PROVENANCE TRIAL IN NORTHEAST IOWA[1]

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ABSTRACT. An investigation of survival and growth of 10 provenances of Scotch pine (Pinus sylvestris L.) was established in northeast Iowa. Approximately 100 2-0 seedlings of each of the 10 seed origins were planted in April 1958 in a 6x6-foot spacing in each of two replications. Based on an evaluation of 5-year survival, height and diameter growth, the seed sources from west central Germany, northern Austria-650m and Czechoslovakia appeared well adapted and grew rapidly on the Fayette silt loam soil type. However, information concerning the bole form of these sources is necessary before their desirability as timber trees can be determined. The Spain source had the best Christmas tree color and form, but its use at this time in northeast Iowa is questionable because of inadequate information regarding adaptability.

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Tree planting failures in Iowa are related to the lack of adequate information on the adaptability of introduced species and to the diversities of climate, soil and topography (Kepler and Gatherum 1964). To minimize tree planting failures, more accurate information is needed on the adaptability to specific sites of species and variants within species.

Scotch pine (Pinus sylvestris L.) has shown promise as a forest tree in species adaptation studies in Iowa. Fast growth rates, tolerance of calcareous soils and resistance to drought make its use desirable on many sites and soil types in Iowa (Jensen and Gatherum 1964). Conversely, poor bole form and early-winter yellowing of foliage of most of the seed sources of Scotch pine previously planted in Iowa have reduced appreciably the value of this species as a timber and Christmas tree.

Because of the wide variation in vigor, growth rate, tree form and foliage color throughout the natural range of Scotch pine (Baldwin 1954, 1956, Lines and Aldhous 1957, Schreiner et al. 1962, Wood 1949, and Wright and Baldwin 1956, 1957) field, greenhouse and laboratory provenance tests have been established to aid in the selection of desirable variants within this species for planting in Iowa.

This 10 seed-source field investigation was established at the Yellow River State Forest in northeast Iowa in 1958. The primary objective was

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to determine the magnitude of the variation in height, diameter, survival and fall-winter foliage color among these 10 Scotch pine seed sources. This information should help improve recommendations for future planting site selection for Scotch pine variants in northeast Iowa and aid in identifying satisfactory genetic material for future breeding and hybridization studies.

MATERIALS AND METHODS

The geographic origin of the 10 seed sources ranged from central Spain, 41°N latitude, to southern Finland, 60°N latitude, encompassing a difference of 19° in latitude and 1,500 meters in elevation. Origin of the seed sources is shown in Figure 1.

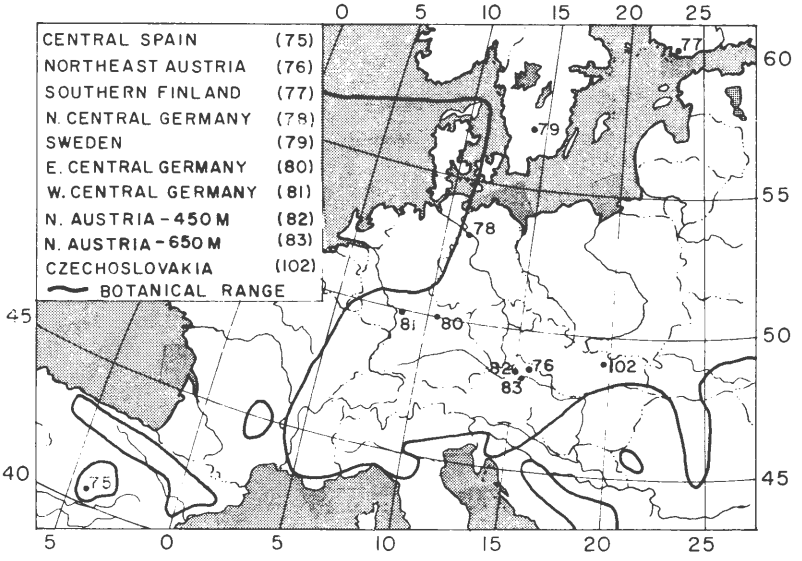

Figure 1. Geographic origin of Scotch pine seed sources used in study. (N. Austria-450m refers to the seed source from northern Austria at an elevation of 450 meters).

The study was conducted at the Paint Creek Unit of the Yellow River State Forest, Allamakee County. The study area is located at a latitude of 43°00'N, a longitude of 91°15'W and an altitude of 320 meters. A pattern of broad ridges and narrow valleys is typical of the general area. The ridges are up to 61 meters high and are broken by small stream channels or an occasional flood plain. Very little level land is present except on the ridge tops and flood plains. Fayette silt loam, a grey-brown podzol, is the soil type of the area. Its parent material is loess from the Iowa till plain (Simonson et al. 1952).

The investigation was established in an open meadow with a N60°E to N60°W aspect and a slope of 12% (Figure 2). The major vegetation consists of Kentucky bluegrass (Poa pratensis L.), red clover (Trifolium pratense L.) and timothy (Phleum pratense L.). The meadow is surrounded by a mixed hardwood forest type of basswood (Tilia americana L.), white ash (Fraxinus americana L.), oaks (Quercus spp. L.), hickories (Carya spp. Nutt.) and elms (Ulmus spp. L.).

Climatic conditions are described as moist subhumid to humid, with a moisture index of 30 (Thornthwaite 1948). The long-time, average annual precipitation is 33.3 inches. The long-time, average yearly maximum, minimum and mean temperatures are 69.7°F, 18.9°F and 48.9°F. The length of the growing season is approximately 140 days. Photoperiod ranges from 15 hours on June 21 to 9 hours on December 21.

Figure 2. General view of the study area at northeast Iowa.

This study was established as a randomized block design according to Cochran and Cox (1957). Approximately 100 2-0 seedlings of each of 10 seed sources were planted in April 1958 in a 6x6-foot spacing in each of two replications. Mortality loss of all except the central Spain and Sweden seed sources was replaced in April 1959. Lack of additional stock for these sources precluded replacement. Five-year survival and height and diameter growth were obtained in August 1962, and relative fall-winter foliage color, crown shape and foliage density were obtained in late November 1962.

RESULTS

Survival varied from 40 to 84%, and differences among several of the seed sources were significant at the 5% or 1% probability level (Figure 3 and Table 1). Survival of the Sweden seed source was lower than survival of the Germany, northern Austria-650m, Czechoslovakia and Finland sources, based on Duncan's multiple range test (Duncan 1955).

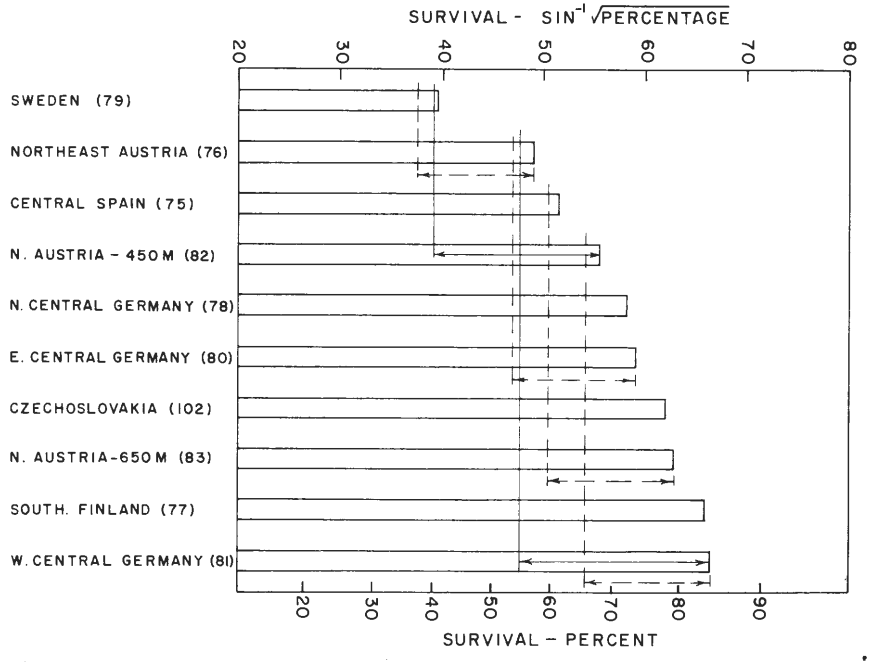

Figure 3. Average 5-year provenance survival. Smallest significant differences, based on Duncan's multiple range test (Duncan 1955), are indicated by arrows: solid line, 1% level; dashed line, 5% level.

Differences in height growth among 10 seed sources ranged from 0.0 to 2.8 feet, and some of these differences were significant at the 1% level (Tables 1 and 2).

No differences were found among the Germany, Austria and Czechoslovakia sources, but this group made more height growth than the Sweden, Finland and Spain sources. Differences among the latter three sources were not significant at the 5% level.

Seed source diameter differences at the ground line ranged from 0.0 to 0.7 inch, and some of these differences were significant at the 1% probability level (Tables 1 and 2). Differences among the Germany, Austria and Czechoslovakia sources and among the Spain, Sweden and Finland sources were not significant at the 5% level. However, the difference between the two groups was significant at the 1% level.

Table 1. Analysis of variance of average 5-year provenance survival and height and diameter growth.

Variation	df	Survival		Height growth		Diam. growth	
		SS	MS	SS	MS	SS	MS
Total	19	1,547.41		19.42		1.33	
Reps.	1	62.69		0.04		0.0003	
Seed Source	9	1,266.82	140.76**	18.72	2.08**	1.25	0.14**
Error A	9	217.90	24.21	0.66	0.07	0.08	0.01

** Significant at 1% level.

Table 2. Average 5-year provenance height growth in feet and diameter growth in inches.

Provenance	Number of observations		Height growth[1]		Diameter growth[1]	
W. central Germany (81)	224		6.2		2.0	
N. central Germany (78)	221		6.0		1.9	
N. Austria-450m (82)	220		5.8		2.0	
N. Austria-650m (83)	223		5.8		2.0	
Czechoslovakia (102)	224	5%	5.8	1%	1.8	5%
E. central Germany (80)	216		5.7		1.9	
Northeast Austria (76)	220		5.6		1.8	
Sweden (79)	78		4.0		1.5	
Southern Finland (77)	224		4.0		1.4	
Central Spain (75)	137		3.4		1.3	

[1] Means grouped by a line do not differ at the designated probability level (Duncan 1955).

Table 3. Relative fall-winter provenance foliage color[1], 5 years after planting.

Source	Quantitative color	Qualitative color
Central Spain (75)	7.5 GY 5/4	Greenish green-yellow
Northeast Austria (76)	2.5 GY 5/4	Yellowish green-yellow
W. central Germany (81)	2.5 GY 5/4	Yellowish green-yellow
N. central Germany (78)	2.5 GY 5/6	Yellowish green-yellow
E. central Germany (80)	2.5 GY 5/6	Yellowish green-yellow
N. Austria-450m (82)	2.5 GY 5/6	Yellowish green-yellow
N. Austria-650m (83)	2.5 GY 5/6	Yellowish green-yellow
Czechoslovakia (102)	2.5 GY 5/6	Yellowish green-yellow
Sweden (79)	2.5 GY 6/6	Yellowish green-yellow
Southern Finland (77)	5.0 Y 5/6	Yellow

[1] Based on Munsell color charts (1952).

Relative fall-winter foliage color, based on Munsell color charts (1952), ranged from greenish-green yellow, 7.5 GY 5/4, of the Spain source to yellow, 5.0 Y 5/6, of the Finland source (Table 3).

DISCUSSION

Average 5-year survival differences among the 10 Scotch pine seed sources were significant at the 5% or 1% probability level. Survival of the west central Germany, southern Finland, northern Austria-650m and the Czechoslovakia sources was highest, but no general relationships between survival and latitudinal origin of seed sources were apparent.

Part of the differences in survival among seed sources was related to establishment problems. Although mortality in the first year was high for most sources, losses in the last 4 years, among the seedlings that survived the first year, were less than 2% for all sources. Furthermore, mortality losses on plots in which seedlings were replaced were less than 5% during the subsequent 4 years. Hence, the initial adaptability of a source cannot be determined definitely until the confounding effect of establishment has been removed.

Average 5-year seed source height and diameter growth differences were significant at the 1% probability level. Height and diameter growth of the sources from central Europe was considerably greater than growth of the sources from the Scandinavian countries. These results corroborate the findings of Lines and Aldhous (1957), Schreiner et al. (1962), Wood (1949) and Wright and Baldwin (1956, 1957).

The slow height and diameter growth of the Spain source, comparable to the growth of the sources from the Scandinavian countries, may be related to selection of a genotype with a slow growth rate, to "browning" of needles on young seedlings in late winter, to movement of the source from a high to a low altitude at the same latitude or to any combination of these. "Browning" of the needles in the nursery and during the first year in the field may have reduced photosynthetic efficiency of the source, thus resulting in slower initial growth for the Spain source than for the central European sources. Introduction of a seed source from high to low altitudes at the same latitude effects environmental changes comparable to the changes resulting from the movement of a source from north to south latitudes. Growth of the Spain source, moved from between 1,000 and 1,500 meters altitude to 320 meters at approximately the same latitude, was similar to growth of the Scandinavian sources, moved south approximately 17° latitude and from less than 100 meters altitude to 320 meters. Movement of the central European sources over a narrower latitudinal and altitudinal range had less influence on growth.

Relative fall-winter foliage color ranged from the yellow of the Finland source to the greenish green-yellow of the Spain source (Munsell color charts 1952), thus corroborating the results of Baldwin (1956) who related yellow foliage color to high latitudinal origin.

From this evaluation of 5-year seedling survival and height and diameter growth, the west central Germany, northern Austria-650m and the Czechoslovakia sources appear to be well adapted and will grow rapidly

on the Fayette silt loam soil type in northeast Iowa. However, information concerning bole form of these sources is necessary before their desirability as timber trees can be determined. Wood (1949) and Wright and Baldwin (1957) observed that the sources of Scotch pine with the poorest bole form came from central Europe.

The Spain source has the most desirable Christmas tree color and form, but the confounding of survival with establishment precludes definite statements concerning adaptability of the Spain source at this time. Hence, additional information on the adaptability of the Spain source to environmental conditions in northeast Iowa must be obtained before definite recommendations concerning its use as a Christmas tree can be made.

SUMMARY

This investigation was established in Allamakee County in northeast Iowa. Approximately 100 2-0 seedlings of each of 10 seed sources were planted in April 1958 in a 6x6-foot spacing in each of two replications. The results of the investigation were:

1. Average 5-year survival ranged from 40 to 84%, and differences among several of the seed sources were significant at the 5 or 1% probability level.

2. Average 5-year height growth ranged from 3.4 to 6.2 feet, and differences among some of the seed sources were significant at the 1% level.

3. Average 5-year diameter growth at the ground line ranged from 1.3 to 2.0 inches, and differences among some of the seed sources were significant at the 1% level.

4. Relative fall-winter foliage color ranged from yellow, 5.0 Y 5/6, for the Finland source to greenish green-yellow, 7.5 GY 5/4, for the Spain source (Munsell color charts 1952).

The west central Germany, northern Austria-650m and Czechoslovakia seed sources appeared well adapted and grew rapidly on the Fayette silt loam soil type in northeast Iowa, but information concerning the bole form of these sources is necessary before their desirability as timber trees can be determined. Moreover, the Spain source had the best Christmas tree color and form, but its use at this time in northeast Iowa is questionable because of inadequate information regarding adaptability.

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ASCORBIC ACID ASSAY BY A SPECIFIC ENZYMATIC METHOD[1,2]

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SUMMARY. A colorimetric procedure is described for determination of L-xyloascorbic acid (vitamin C) in microgram amounts. It depends on the specificity of an ascorbic acid oxidase from spores of Myrothecium verrucaria and is based on the difference in indophenol reducing power with and without prior enzymatic oxidation. L-Xyloascorbate is differentiated from reductone, reductate, and certain other enediols. D-Araboascorbate interferes apparently by inactivating the enzyme.

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One of the difficulties in studying the biological role and metabolism of ascorbic acid is lack of a highly specific analytical method. The two most widely used methods are based on indophenol dye titration or on colorimetric determination of osazones of oxidized ascorbate (3). Tillman's dye method, in spite of various modifications, still is subject to interference by many reducing substances. Roe and Keuther's osazone method avoids this difficulty but does not differentiate between L-xyloascorbate, the naturally-occurring antiscorbutic compound, and its structural isomers or closely-related enediols.

Some improvement in specificity with the indophenol method was achieved by Srinivasan (4) and by Meiklejohn and Stewart (2) with the use of ascorbic acid oxidase. Their procedures were based on the decrease in reducing power of solutions after enzymatic oxidation. Unfortunately, the higher plant oxidase has a sufficiently broad substrate specificity (5) so this modification of the dye method also does not adequately differentiate ascorbate analogs.

In recent work in our laboratory, the ascorbic acid oxidase of Myrothecium verrucaria was tested on 22 ascorbate analogs. Only L-xyloascorbate was oxidized at a rate detectable in the Warburg (5). This paper reports progress in devising a colorimetric procedure for microgram amounts of L-xyloascorbate based on this specificity of the fungal enzyme.

[1] Received December 2, 1963.

[2] These studies were aided by a Public Health Service Research Grant, GM-7354.

MATERIALS

The culture of Myrothecium verrucaria (Alb. and Schw.) Dit. ex Fr. (strain QM-460) was obtained in 1949 from the Quartermaster General Laboratories. The sources of the ascorbic acid analogs were as follows: L-xyloascorbic acid, (hereafter, L-ascorbic acid)—Merck & Co.; D-araboascorbic acid—Hoffman LaRoche; reductone—Dr. J. R. Holker, Tootal Broadhurst Lee Co., Manchester, Eng.; reductic acid and methyl-reductic acid—Dr. G. Hesse, U. of Freiburg, Ger.; 5,6-diacetyl-L-xyloascorbic acid—Dr. W. Feldheim, Inst. Für Ernährung, Deutsch. Akad. der Wissen. zu Berlin; L-glucoascorbic acid—synthesized by a modification of Helferich's benzoin condensation procedure (1).

ENZYME PREPARATION

Spores were produced in cultures on filter paper supported on a solid agar-salts medium in petri dishes (5). Spores from week-old cultures were brushed into water suspension, washed by centrifugation, and stored at -20°C. The average yield was about 0.6 ml centrifuge-packed spores per dish at about 20% dry weight (w/v). The frozen spore suspension maintained adequate oxidase activity for at least 6 months (Table I).

Table I. Effect of spore storage at -20°C on oxidase activity.

Spore lot	Storage time (days)	Oxidase units* per mg dry wt of spores
11-27-1	10	4.7
	105	3.8
6-1-2	19	3.7
	196	4.4
1-31-3	5	4.4
	45	3.9

*Oxidase units: μg ascorbate oxidized per min. per ml reaction mixture (initial rate).

Extraction of the enzyme in the Mickle disintegrator and all subsequent operations were carried out at 0-5°C. The grinding mixture per tube consisted of 3 ml of spore suspension, 5 g No.12 Ballotini beads, 1.5 g of No.600 carborundum power, and water to make a liquid volume of 7.5 ml. Shaking for 30 min. at an amplitude of 1-2 cm gave at least 95% spore rupture by microscopic examination. The homogenate was washed into a centrifuge tube with 4 ml of water, spun at 2000 x g. for 5 min. and the supernatant decanted. The sediment was resuspended with another 4 ml of water, recentrifuged, and the supernatants combined. The extract was then centrifuged at 30,000 x g. for 30 min. and the high speed supernatant was stored frozen at -20°C. Enzyme activity was maintained for at least a month and in some cases considerably longer (Table II).

Table II. Effect of storage at -20°C on oxidase activity of spore extracts.

Enzyme prep.	Spore lot	Storage time (at -20°C)	Activity * (OU/ml)
M8	10-21-1	0	163
		19	200
M10	11-27-1	0	197
		43	135
M11	"	0	252
		37	224
M17	6-1-2	2	154
		40	126
M18	"	0	147
		116	105

*Oxidase units: μg ascorbate oxidized per min. per ml reaction mixture (initial rate).

METHOD OF ANALYSIS

The method was based on colorimetric determination of total reducing power of the solution to be assayed on one aliquot and of the reducing power remaining after enzymatic oxidation on another. The difference provided a measure of the L-ascorbic acid content.

Procedure for the standard enzymatic reaction. The reaction vessel was a 40 ml conical centrifuge tube immersed in a 30°C bath. Oxygen saturated with water vapor was bubbled through continuously to keep the reaction mixture saturated. The reaction mixture contained 2 ml of the solution to be assayed, 1.2 ml of 0.067 M phosphate-0.032 M citrate at pH 6, and 0.8 ml of the enzyme extract. The assay solution contained 5×10^{-5} M EDTA and 3% metaphosphate to stabilize the reducing substances and was adjusted to pH 6 and to a reducing power equivalent to 25-50 μg of ascorbic acid. Enzyme activity was adjusted to give complete oxidation of 50 μg of ascorbic acid in from 5 to 10 min. Two 1 ml samples of the reaction mixture were taken for colorimetric analysis, the first at a time when the reaction should be complete based on prior enzyme standardization, the second 2 or 3 min. later to confirm completion.

Colorimetric analysis of reducing power. The 1 ml samples of reaction mixture were pipetted into 10 ml test tubes containing 0.5 ml of 15 M acetate buffer at pH 4. Next, 1 ml of 3×10^{-4} M, 2,6-dichlorophenolindophenol in 0.02% sodium bicarbonate was added. If ascorbate analogs not oxidized by the enzyme were present, part of the dye was reduced instantaneously. Excess oxidized dye then was extracted by

adding 5 ml of distilled xylene and shaking for 10 sec. The dye was stable for several hours in the xylene layer. The tubes were centrifuged to clarify the xylene, and the latter was pipetted into matched colorimeter tubes. Absorbancies were read at 510 mμ in a Photovolt colorimeter against xylene blanks.

To get an accurate measure of the reducing power of enzyme reaction samples it generally was necessary to make a correction for retention of oxidized dye in the aqueous phase after xylene extraction. This can cause a significant decrease in the dye absorbancy of the xylene layer. A satisfactory correction was made on the basis that dye retention is proportional to the oxidized dye concentration in the xylene over the range of concentrations used in the method. This proportionality constant must be determined for each batch of enzyme extract.

The total reducing power of the assay solution was determined in identical fashion with a second aliquot except that water was substituted for enzyme extract. Again, two samples were taken from the reaction mixture at different times, in this case to establish that no significant autoxidation occurred.

From the difference in dye absorbance of the two samples with and without prior enzymatic oxidation, the concentration of L-ascorbic acid in the original solution was calculated using a dye standardization value obtained with pure ascorbate.

FACTORS AFFECTING THE ENZYMATIC OXIDATION

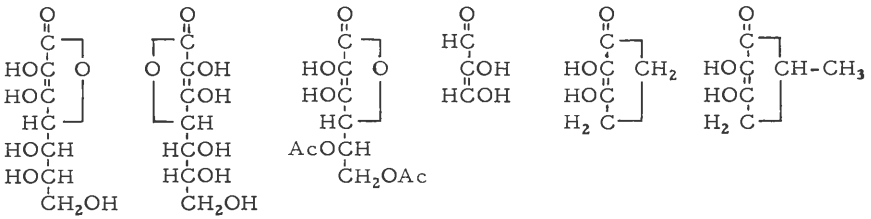
In working out this procedure several factors which might affect the enzymatic reaction or the stability of ascorbate were investigated. Metaphosphate at 3% was included in the assay solutions since we anticipated using it as an extractant in analysis of ascorbic acid in tissues. Under the standard reaction conditions this concentration of metaphosphate did not alter enzyme activity. A pH of 6 was chosen since the enzyme was more active than at lower pH's yet ascorbate was still sufficiently stable. It was necessary, however, to include 2.5×10^{-5} M EDTA in the reaction mixture to prevent autoxidation of L-ascorbic acid and its analogs. Although previous manometric work had shown the enzyme was sensitive to EDTA (6), the loss in activity was not significant under our assay conditions, and EDTA reduced autoxidation to a negligible level (Table III). Finally, since this enzyme has a relatively low affinity for oxygen, it was possible to reduce the reaction time to about half by bubbling pure oxygen through the reaction mixture (Fig. 1).

Table III. Effect of EDTA (2.5×10^{-4} M) on L-ascorbate oxidation.

Reaction conditions	Ascorbate oxidized (μg/ml) 1 min.	10 min.
With enzyme	24.5	50
With enzyme, with EDTA	24.0	50
Without enzyme	0	1.5
Without enzyme, with EDTA	0	0

Table IV. Determinations of L-ascorbic acid in presence of certain analogs.

	Analog (μg/ml, L-ascorbate equivalent)		L-Ascorbate (μg/ml) Added	Found
I	L-Glucoascorbate	21.5	25	25.5
	"	23	25	26
II	D-Glucoascorbate	24	25	25.5
	"	23	25	26
III	Diacetyl-L-asc.	22	25	25.5
	"	20.5	25	24.5
IV	Reductone	22	25	25
	"	22	25	24.5
V	Reductate	25	25	24.5
	"	24	25	24.5
VI	Methylreductate	22	25	24.5
	"	22.5	25	26



I II III IV V VI

ANALYSIS OF L-ASCORBIC ACID IN PRESENCE OF ANALOGS

This enzymatic procedure has been tested principally on the 6 analogs of L-ascorbic acid shown in Table IV. All of these compounds have an enediol grouping like L-xyloascorbate and can not be satisfactorily differentiated from it by any other methods short, perhaps, of chromatographic separation or bioassay. In the experiments summarized, a mixture of 20-25 μg of L-ascorbic acid with a similar amount of one of the analogs was assayed. The results indicate that one can determine L-ascorbate with an accuracy of about ± 1 μg or about 4% under these conditions.

The enzyme does have slight activity toward some of these analogs under the standard reaction conditions, and a correction must be applied

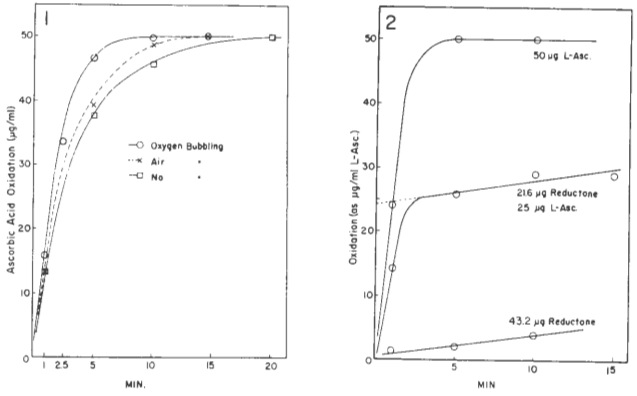


Figure 1. L-Ascorbic acid oxidized as a function of reaction time under standard conditions with varying oxygen supply.

Figure 2. L-Ascorbic acid oxidized as a function of time under standard reaction conditions in presence of reductone. All concentrations are expressed as µg/ml of L-ascorbic acid.

as indicated for reductone in Fig. 2. L-Ascorbate at concentrations up to 50 µg/ml was fully oxidized in about 5 min. Reductone at a similar concentration was oxidized at a rate of about 2 µg/ml in the same time. In the mixture there was initial rapid oxidation of L-ascorbate followed by slow oxidation of reductone. Adequate correction for the latter was made by extrapolation to zero time. Similar oxidation curves and the correction for L-ascorbate oxidation in presence of L-glucoascorbate are shown in Fig. 3.

The only analog tested for which the method failed is D-araboascorbate, the C-5 epimer of L-ascorbate. As indicated in Fig. 4, the rate of oxidation of this analog was fairly rapid for a minute or two and then fell off. In the mixture, complete oxidation of L-ascorbate did not occur even after 60 min. indicating that enzyme activity was inhibited by D-araboascorbate. In other experiments irreversible inhibition was demonstrated by incubation of the enzyme with D-ascorbate, dialysis, and then testing with L-ascorbate.

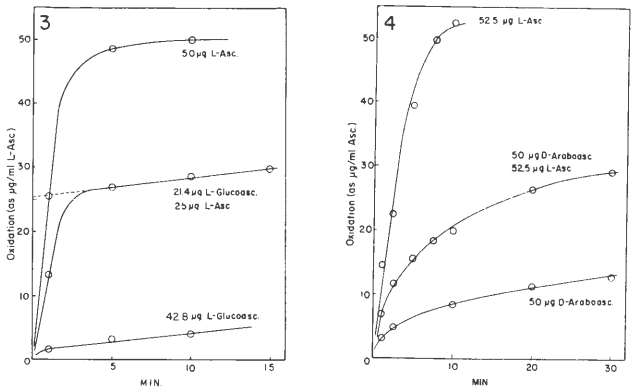


Figure 3. L-Ascorbic acid oxidized as a function of time under standard reaction conditions in presence of L-glucoascorbate. All concentrations are expressed as µg/ml of L-ascorbic acid.

Figure 4. Oxidation of L-ascorbic and D-araboascorbic acids, separately and mixed, as a function of time under standard reaction conditions. All concentrations are expressed as µg/ml of L-ascorbic acid.

CONCLUSION

The method described is applicable to analysis of L-ascorbate in the presence of certain enediol analogs not adequately differentiated by other analytical methods. It failed with D-araboascorbate due to inhibition of the enzyme. Further work with other analogs, now in preparation, is necessary before the nature of this inhibition and its restriction on the method can be determined. The method may be especially valuable for determining L-ascorbate in the presence of reductone, reductate, and similar carbohydrate degradation products which occur in foodstuffs.

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JAPANESE LARCH PROVENANCE TRIAL IN NORTHEAST IOWA[1]

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ABSTRACT. An investigation of survival and growth of seven provenances of Japanese larch (Larix leptolepis (Sieb. and Zucc.) Gord.) was established in Allamakee County, Iowa. Four 2-0 seedlings of each of seven seed origins from Japan were planted in April 1960 at an 8x8-foot spacing in each of ten replications. An evaluation of 3-year-old seedling data has shown high survival of all provenances and good growth of provenances 153, Gumma prefecture; and 156, Nagano prefecture. A positive correlation of average height and diameter growth with increasing altitudinal origin of seed source may be related to the introduction of these sources from 36° N latitude in Japan to 43° N latitude in Iowa.

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By 1961, a total of 83,515 acres of forest plantations and windbarriers had been planted in Iowa (USDA 1961). The problems of selecting the proper species or variants within species for each planting site have been acute because of the diversities of climate, soil and topography and the frequent use of introduced tree species (Bode 1921, Dilworth 1938, Einspahr and McComb 1951, Eschner 1952, Iowa State University 1960, Simonsen et al. 1952). Tree planting guides for timber, windbarriers, erosion control and wildlife cover have been established from limited studies and observations made in the state (Iowa State College 1957). However, to improve planting recommendations, more accurate information is needed on the adaptability to specific sites of species and variants within species.

[1] Journal Paper No. J-4639 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. Project No. 1482. Contribution from Iowa Agricultural and Home Economics Experiment Station as a collaborator under North Central Region cooperative research project titled, "Forest Tree Improvement Through Selection and Breeding." All Japanese larch seedlings used in this study were provided by Dr. J.W. Wright, Department of Forestry, Michigan State University.

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[3] Associate Professor, Department of Forestry, Iowa State University.

Such information is needed for the proper use of Japanese larch (Larix leptolepis (Sieb. and Zucc.) Gord.) in converted stands, plantations and windbarriers in Iowa. Japanese larch potential for future planting appears high because of its rapid growth (Aird and Stone 1955, Cook 1942, Pruett[4]) and good stem form (Littlefield and Eliason 1956, Wood et al. 1960). However, its native altitudinal range, from 915 to 2,744 meters above sea level (Streets 1962), encompasses environmental differences which have effected marked variation within species through natural selection (Day 1946, Edwards and Pinchin 1953, Langner 1960, 1961, Lindquist 1956, Wachter 1961, Wood et al. 1960). Variation in stem form, growth rate and other physiological and morphological characters has been well documented (Anderson 1950, Cook 1960, Day 1946, Day and Pease 1935, Douglass 1961, Edwards and Pinchin 1953, Gathy 1957, Laing 1944, Langner 1960, 1961, Leven 1951, Lindquist 1956, Macdonald et al. 1957, Matthews 1954, Paton 1944, Robinson 1931, Schrober 1958, Wachter 1961, Wood and Edwards 1954, Wood et al. 1960, Wright and Genys in Genys 1960, Zehetmayer 1960).

To aid in the identification and evaluation of this within-species variation, a seven seed-source investigation was established in northeast Iowa in 1960. The objectives of this study were (1) to determine the magnitude of the height, diameter and survival variation among seven seed sources of Japanese larch, (2) to aid in the improvement of recommendations for future planting site selection for Japanese larch and its variants in northeast Iowa, and (3) to aid in the ultimate identification of satisfactory breeding material for future breeding and hybridization studies.

METHODS AND PROCEDURES

The origin of the seven seed sources ranged in Japan from 35°24' to 36°47'N latitude, 137° 34' to 139° 32'E longitude and 1,400 - 2,000 meter altitude (Table 1).

The study was conducted at the Paint Creek Unit of the Yellow River State Forest, Allamakee County. The study area is located at a latitude of 43°00'N, a longitude of 91°15'W and an altitude of 320 meters. A pattern of broad ridges and narrow valleys is typical of the general area. The ridges are up to 61 meters high and are broken by small stream channels or an occasional flood plain. Very little level land is present except on the ridge tops and flood plains. Fayette silt loam, a grey-brown podzol, is the soil type of the area. Its parent material is loess from the Iowa till plain (Simonson et al. 1952).

The investigation was established in an open meadow with a N60°W aspect and a slope of 12% (Fig. 1). The major vegetation consists of Kentucky bluegrass (Poa pratensis L.), red clover (Trifolium pratense L.) and timothy (Phleum pratense L.). The meadow is surrounded by a mixed hardwood forest type of basswood (Tilia americana L.), white ash (Fraxinus americana L.), oaks (Quercus spp. L.), hickories (Carya spp. Nutt.) and elms (Ulmus spp. L.).

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4 Pruett, Emerson, Amana, Iowa. 1962. Data from northeast Iowa experimental plantations. Private communication.

Table 1. Origin of Japanese larch provenances from Japan.

Provenance number		Prefecture	Village	Altitude (meters)	Latitude N. o	'	Longitude E. o	'
152[1]	(1)[2]	Tochigi	Yasyubara N.F.	1,700	36	47	139	32
153	(2)	Gumma	Kumashiro N.F.	1,700-1,800	36	28	138	29
154	(3)	Nagano	Azusayama	1,500	35	36	138	41
155	(4)	Nagano	Nishidake N.F.	1,450	35	36	138	19
156	(5)	Nagano	Hontaniyama N.F.	2,000	35	27	138	06
157	(6)	Nagano	Takasegawa N.F.	1,400	36	24	137	41
158	(7)	Nagano	Mitake N.F.	1,400	35	54	137	34

The following footnotes are intended for all tables and figures giving provenance numbers of investigation.

[1] Iowa State University, Department of Forestry number.

[2] Michigan State University, Department of Forestry number.

Figure 1. General view of the experimental site.

Climatic conditions are described as moist subhumid to humid, with a moisture index of 30 (Thornthwaite 1948). The long-time average annual precipitation is 33.3 inches. The long-time average yearly maximum, minimum and mean temperatures are 69.7°F, 18.9° F and 48.9°F. The length of the growing season is approximately 140 days. Photoperiod ranges from 15 hours on June 21 to 9 hours on December 21.

This study was established as a randomized block design according to Cochran and Cox (1957). Four 2-0 seedlings of each of the seven seed sources of Japanese larch were planted in April 1960 at 8 x 8-foot spacing in each of ten replications. Mortality loss was replaced in April 1961 by 3-0 seedlings. Three-year survival and height and diameter growth were obtained in August 1962.

RESULTS

Survival among the seven provenances varied from 86 to 95% (Table 2), and differences were not significant at the 5% probability level (Table 3).

Height growth differences among the seed sources ranged from 0.0 to 1.4 feet (Table 2), and differences equal to or greater than 0.9 and 1.2 feet were significant at the 5 and 1% probability levels (Tables 2 and 3). At the 5% level, height growth of provenance 153, Gumma prefecture, was greater than the height growth of provenances 155, 158, 154 and 157; and height growth of provenance 156, Nagano prefecture, was greater than height growth of provenances 154 and 157 (Table 2).

Table 2. Average 3-year provenance survival in percent, height growth in feet and diameter growth in inches.

Provenance number	Prefecture	Altitude (meters)	Survival	Average height growth[1]	Average diameter growth[1]
153 (2)	Gumma	1700-1800	94	5.4	1.0
156 (5)	Nagano	2000	94	5.2	1.0
152 (1)	Tochigi	1700	95	4.9	0.9
155(4)	Nagano	1450	94	4.5	0.9
158 (7)	Nagano	1400	91	4.5	0.8
154 (3)	Nagano	1500	90	4.3	0.7
157 (6)	Nagano	1400	86	4.0	0.7

5% 1% 5%

[1] Means grouped by a line do not differ at the designated probability level (Duncan 1955).

Table 3. Analysis of variance of average 3-year survival, height growth and diameter growth.

Source	df	Total 70	Replication 9	Provenance 6	Error 54
Survival					
SS		15,779.60	3,506.34	876.02	11,397.24
MS			389.59	146.00	211.06
F			1.84	0.69	
Height Growth					
SS		64.38	6.14	15.58	42.66
MS			0.68	2.60	0.79
F			0.86	3.29**	
Diameter Growth					
SS		4.51	0.30	1.29	2.92
MS			0.03	0.22	0.054
F			0.06	4.07**	

** Significant at 1% probability level.

Provenance differences in diameter growth at ground level ranged from 0.0 to 0.3 inch (Table 2). Differences equal to or greater than 0.2 and 0.3 inch were significant at the 5 and 1% probability levels (Tables 2 and 3). At the 5% level, diameter growth of provenances 153, Gumma prefecture and 156, Nagano prefecture was higher than diameter growth of provenances 158, 154 and 157 (Table 2).

Three-year growth of individual trees ranged from 1.6 feet in height and 0.2 inch in diameter, provenance 154, Nagano prefecture, to 7.3 feet in height and 1.6 inches in diameter, provenance 156, Nagano prefecture (Figs. 2, 3).

Analyses were made to determine the relation between height and diameter growth and altitudinal origin of each seed source. Average height and diameter growth were correlated positively with altitudinal origin, and the correlation coefficients were significant at the 1% level. The correlations of average height and diameter growth with altitudinal origin accounted for 72 and 77% of the variation (Figs. 4, 5).

DISCUSSION

Average 3-year survival of the seven seed sources of Japanese larch planted in northeast Iowa was high (Table 2). The results contrast with the low survival obtained by Pruett[4] on the same sites with one source of Japanese larch of different origin. The difference probably reflects, in part, the variation in specific provenance adaptability in northeast Iowa. Moreover, the difference lends credence to Pruett's suggestion that within-species trials may lead to better-adapted sources and the rapid rate of growth identified previously. Apparently, freezing and snow damage resistance among the seven seed sources was greater than the damage resistance observed by Hansen[5] in Pruett's study.

Differences in average 3-year provenance height and diameter growth were highly significant (Tables 2 and 3), and individual tree height and diameter growth within provenances was extremely variable (Figs. 2, 3). These results corroborate earlier reports of Wright and Genys in Genys (1960) on the same provenances in Michigan.

The consistent differences among provenances in height and diameter growth (Table 2) and the uniformly high survival of all seed sources facilitate selection of seed origins best adapted in terms of seedling survival and growth. Provenances 153 and 156, originating from altitudes greater than 1,700 meters, generally attained good growth (Table 2). The positive correlation of average height and diameter growth with increasing altitudinal origin of seed source (Figs. 4, 5), may be related to the introduction of these sources from 36°N latitude in Japan to 43°N latitude in Iowa. Pauley (1957) hypothesizes increased growth through matching of longer photoperiods-shorter growing seasons of northern locations to the shorter growing season adaptation of high altitude, southern seed sources.

[5] Hansen, Norman, Drakesville, Iowa. 1961. Data from Forestry Section of Iowa Agricultural and Home Economics Experiment Station. Private communication.

Figure 2. Smallest 3-year height and diameter growth of 1.6 feet and 0.2 inch was attained by an individual seedling of provenance 154, Nagano prefecture. Average 3-year height and diameter growth for this provenance was 4.3 feet and 0.7 inch.

Figure 3. Greatest 3-year height and diameter growth of 7.3 feet and 1.6 inches was attained by an individual seedling of provenance 156, Nagano prefecture. Average 3-year height and diameter growth for this provenance was 5.2 feet and 1.0 inch.

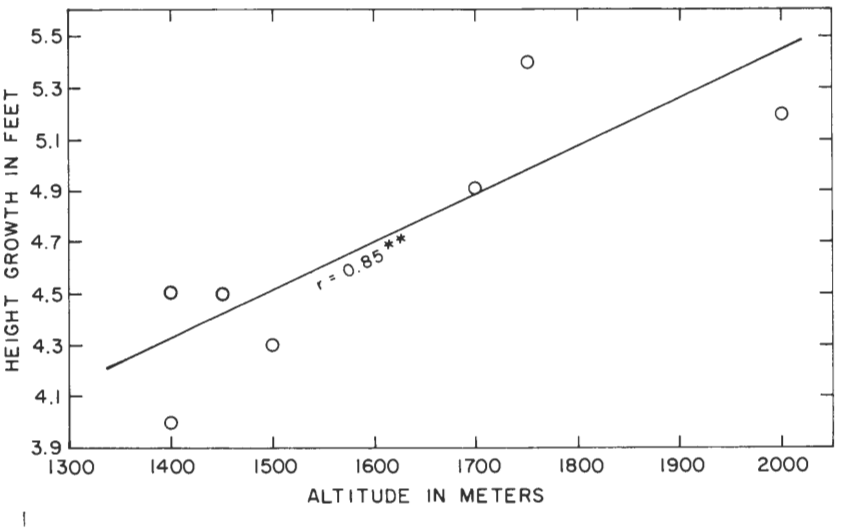

Figure 4. Correlation of average 3-year height growth of seedlings with altitudinal origin of seed source.

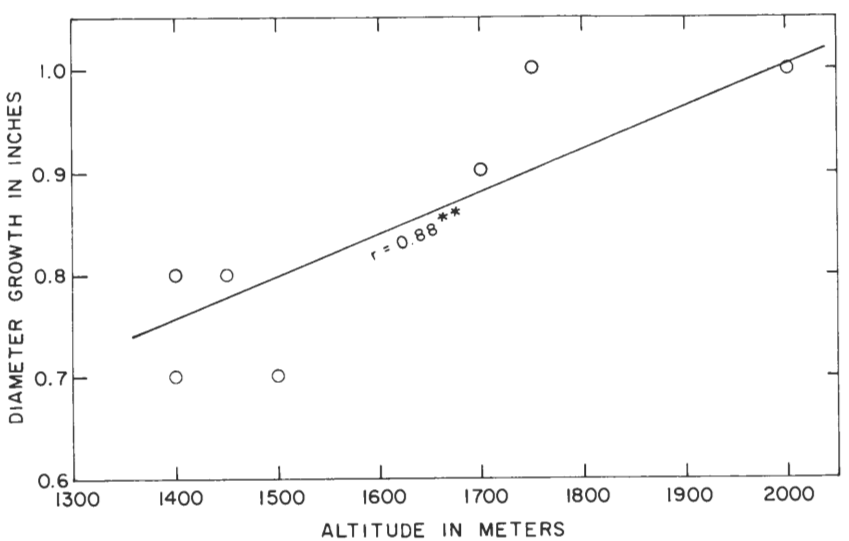

Figure 5. Correlation of average 3-year diameter growth of seedlings with altitudinal origin of seed source.

SUMMARY

An investigation of survival and growth of seven provenances of Japanese larch was established in Allamakee County, Iowa. Four 2-0 seedlings of each of the seven seed origins from Japan were planted in April 1960 at an 8x8-foot spacing in each of ten replications.

The results were:

1. Average 3-year survival was high, ranging from 86 to 95%, and differences among provenances were not significant at the 5% probability level.
2. Average 3-year height growth ranged from 4.0 to 5.4 feet and differences among provenances were significant at the 1 or 5% level.
3. Average 3-year diameter growth ranged from 0.7 to 1.0 inch, and differences among provenances were significant at the 1 or 5% level.
4. Average 3-year height and diameter growth of provenances 153, Gumma prefecture and 156, Nagano prefecture was high.
5. Individual tree growth ranged from 1.6 feet in height and 0.2 inch in diameter, provenance 154, Nagano prefecture, to 7.4 feet in height and 1.6 inches in diameter, provenance 156, Nagano prefecture.
6. Average height and diameter growth was correlated positively with altitudinal origin of seed source, and the correlation coefficients were significant at the 1% level.

An evaluation of the 3-year-old seedling data has shown high survival of all provenances and good growth of provenances 153, Gumma prefecture and 156, Nagano prefecture. The high level of adaptability and the relatively rapid growth of these two sources indicate, at least for the first 3 years, that some seed sources of Japanese larch will survive and grow rapidly on Fayette silt loams in northeast Iowa. A positive correlation of average height and diameter growth with increasing altitudinal origin of seed source may be related to the introduction of these sources from 36°N latitude in Japan to 48°N latitude in Iowa.

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